8/750101

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PASSWORD:
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FILE 'HOME' ENTERED AT 11:54:03 ON 15 MAR 1998

=> uspatfull

USPATFULL IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
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=> e dolly, james oliver/ai

THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
The EXPAND command is used to look at the index in a file
which has an index.  This file does not have an index.

=> e dolly james oliver/au

THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
The EXPAND command is used to look at the index in a file
which has an index.  This file does not have an index.

=> e dolly james oliver/in

THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
The EXPAND command is used to look at the index in a file
which has an index.  This file does not have an index.
```

```
=> uspatfull

USPATFULL IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s colistridal (5a) toxin?

THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
Some commands only work in certain files.  For example, the EXPAND
command can only be used to look at the index in a file which has an
index.  Enter "HELP COMMANDS" at an arrow prompt (=>) for a list of
commands which can be used in this file.

=> file uspatfull

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.60       0.60

FILE 'USPATFULL' ENTERED AT 11:56:13 ON 15 MAR 1998
CA INDEXING COPYRIGHT (C) 1998 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 10 Mar 1998 (19980310/PD)
FILE LAST UPDATED: 11 Mar 1998 (19980311/ED)
HIGHEST PATENT NUMBER: US5727249
CA INDEXING IS CURRENT THROUGH 11 Mar 1998 (19980311/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 10 Mar 1998 (19980310/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: JAN 1998
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: DEC 1997

>>> Page images are available for patents from 1/1/95.  Current     <<<
>>> week patent text is typically loaded by Thursday morning and    <<<
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>>> is included in file records.  A thesaurus is available for the  <<<
>>> USPTO Manual of Classifications in the /NCL, /INCL, and /RPCL   <<<
>>> fields.  This thesaurus includes catchword terms from the       <<<
>>> USPTO/MOC subject headings and subheadings.  Thesauri are also  <<<
>>> available for the WIPO International Patent Classification      <<<
>>> (IPC) Manuals, editions 1-6, in the /IC1, /IC2, /IC3, /IC4,     <<<
>>> /IC5, and /IC (/IC6) fields, respectively.  The thesauri in     <<<
>>> the /IC5 and /IC fields include the corresponding catchword     <<<
>>> terms from the IPC subject headings and subheadings.            <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> e dolly james oliver/au

E1            1     DOLLOFF RICHARD T/AU
E2            2     DOLLST KLAUS/AU
E3            0 --> DOLLY JAMES OLIVER/AU
E4            1     DOLLYHIGH SAMUEL M/AU
E5            1     DOLMAN DENVER L/AU
E6            1     DOLMAN GERRI/AU
E7           10     DOLMAN HENDRIK/AU
E8            1     DOLMAN JAMES/AU
E9            1     DOLMAN JOHN E/AU
E10           1     DOLMAN KEVIN F/AU
E11           2     DOLMAN RODNEY A/AU
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E12          2     DOLMAN ROY S/AU

=> e aoki kei roger/au

E1           2     AOKI KAZUYOSHI/AU
E2          30     AOKI KEI/AU
E3           1 --> AOKI KEI ROGER/AU
E4           1     AOKI KEICHIRO/AU
E5           1     AOKI KEIGO/AU
E6           5     AOKI KEIICHI/AU
E7           3     AOKI KEIICHIRO/AU
E8          39     AOKI KEIJI/AU
E9           4     AOKI KEN/AU
E10          4     AOKI KEN ICHI/AU
E11          5     AOKI KENICHI/AU
E12         24     AOKI KENJI/AU

=> s e2 or e3

            30 "AOKI KEI"/AU
             1 "AOKI KEI ROGER"/AU
L1          31 "AOKI KEI"/AU OR "AOKI KEI ROGER"/AU

=> s l1 and toxin?

          7082 TOXIN?
L2           1 L1 AND TOXIN?

=> d bib ab


L2   ANSWER 1 OF 1  USPATFULL
AN     1998:19686  USPATFULL
TI     Injectable therapy for control of muscle spasms and pain related
       to muscle spasms
IN     Aoki, Kei Roger, Laguna Hills, CA, United States
       Wheeler, Larry A., Irvine, CA, United States
       Garst, Michael E., Newport Beach, CA, United States
PA     Allergan, Waco, TX, United States (U.S. corporation)
PI     US 5721215  980224
AI     US 96-619780  960320 (8)
DT     Utility
EXNAM  Primary Examiner: Walsh, Stephen; Assistant Examiner: Basham,
       Daryl A.
LREP   Hackler, Walter A.
CLMN   Number of Claims: 18
ECL    Exemplary Claim: 8
DRWN   42 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 858
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     A method for administration of botulinum toxin, includes
       the steps of (a) selecting at least one neuromuscular blocking
       agent having a duration of activity shorter than neuromuscular
       blocking activity of botulinum toxin; (b) selecting at
       least one muscle of a muscle group; (c) intramuscularly injecting
       the selected agent into the selected muscle; (d) observing muscle
       relaxation in both the selected muscle and other nonselected
       muscles in the muscle group to determine spill-over, muscle tone
       and balance; (e) repeating steps (b)-(d) until a final muscle
       selection is found; and (f) intramuscularly injecting botulinum
     toxin into the final muscle selection.

=> e garst michael elwood/au

E1           3     GARST JOHN M/AU
```

```
E2          40      GARST MICHAEL E/AU
E3           0 --> GARST MICHAEL ELWOOD/AU
E4           1      GARST MICHAEL G/AU
E5           1      GARST ORVILLE L/AU
E6           5      GARST ROGER H/AU
E7           2      GARSTANG JAMES H/AU
E8           1      GARSTANG WILLIAM W/AU
E9           1      GARSTEN CARL JOHAN/AU
E10          1      GARSTICK GEORGE A/AU
E11          1      GARSTICK LARRY A/AU
E12          1      GARSTIN DAVID JOHN IVOR/AU

=> s e2

L3          40 "GARST MICHAEL E"/AU

=> s l3 and toxin?

          7082 TOXIN?
L4           1 L3 AND TOXIN?

=> d bib ab


L4   ANSWER 1 OF 1  USPATFULL
AN     1998:19686  USPATFULL
TI     Injectable therapy for control of muscle spasms and pain related
       to muscle spasms
IN     Aoki, Kei Roger, Laguna Hills, CA, United States
       Wheeler, Larry A., Irvine, CA, United States
       Garst, Michael E., Newport Beach, CA, United States
PA     Allergan, Waco, TX, United States (U.S. corporation)
PI     US 5721215  980224
AI     US 96-619780  960320 (8)
DT     Utility
EXNAM  Primary Examiner: Walsh, Stephen; Assistant Examiner: Basham,
       Daryl A.
LREP   Hackler, Walter A.
CLMN   Number of Claims: 18
ECL    Exemplary Claim: 8
DRWN   42 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 858
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     A method for administration of botulinum toxin, includes
       the steps of (a) selecting at least one neuromuscular blocking
       agent having a duration of activity shorter than neuromuscular
       blocking activity of botulinum toxin; (b) selecting at
       least one muscle of a muscle group; (c) intramuscularly injecting
       the selected agent into the selected muscle; (d) observing muscle
       relaxation in both the selected muscle and other nonselected
       muscles in the muscle group to determine spill-over, muscle tone
       and balance; (e) repeating steps (b)-(d) until a final muscle
       selection is found; and (f) intramuscularly injecting botulinum
     toxin into the final muscle selection.

=> e dolly james/au

E1           1      DOLLOFF RICHARD T/AU
E2           2      DOLLST KLAUS/AU
E3           0 --> DOLLY JAMES/AU
E4           1      DOLLYHIGH SAMUEL M/AU
E5           1      DOLMAN DENVER L/AU
E6           1      DOLMAN GERRI/AU
E7          10      DOLMAN HENDRIK/AU
E8           1      DOLMAN JAMES/AU
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E9            1     DOLMAN JOHN E/AU
E10           1     DOLMAN KEVIN F/AU
E11           2     DOLMAN RODNEY A/AU
E12           2     DOLMAN ROY S/AU

=> s clostritdal (5a) toxin?

              0 CLOSTRITDAL
           7082 TOXIN?
L5            0 CLOSTRITDAL (5A) TOXIN?

=> s clostridial (5a) toxin?

             97 CLOSTRIDIAL
           7082 TOXIN?
L6            8 CLOSTRIDIAL (5A) TOXIN?

=> s l6 and (conjugat? or fusion or link? or fused)

          45443 CONJUGAT?
          39343 FUSION
         346086 LINK?
          67646 FUSED
L7            6 L6 AND (CONJUGAT? OR FUSION OR LINK? OR FUSED)

=> d bib ab 1-6


L7   ANSWER 1 OF 6  USPATFULL
AN     1998:17427  USPATFULL
TI     Clostridial toxin disease therapy
IN     Carroll, Sean B., Cottage Grove, WI, United States
       van Boldrik, Margaret B., Cottage Grove, WI, United States
       Clemens, Christopher M., Madison, WI, United States
PA     Ophidian Pharmaceuticals Inc., Madison, WI, United States (U.S.
       corporation)
PI     US 5719267  980217
AI     US 95-457890  950601 (8)
RLI    Division of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a
       continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct
       1989, now patented, Pat. No. US 5196193
DT     Utility
EXNAM  Primary Examiner: Eisenschenk, Frank C.
LREP   Medlen & Carroll,LLP
CLMN   Number of Claims: 10
ECL    Exemplary Claim: 1
DRWN   2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1310
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Treating humans and animals intoxicated with a bacterial toxin by
       administration of antitoxin. Avian antitoxin in an aqueous
       solution in therapeutic amount that is orally administrable.

L7   ANSWER 2 OF 6  USPATFULL
AN     97:115238  USPATFULL
TI     Pharmaceutical composition containing botulinum B complex
IN     Johnson, Eric A., Madison, WI, United States
       Goodnough, Michael C., Madison, WI, United States
       Borodic, Gary E., Canton, MA, United States
PA     Associated Synapse Biologics, Cambridge, MA, United States (U.S.
       corporation)
PI     US 5696077  971209
AI     US 94-316820  941003 (8)
RLI    Continuation of Ser. No. US 93-140328, filed on 20 Oct 1993, now
       abandoned
```

DT Utility
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Wai, Thanda
LREP Testa, Hurwitz & Thibeault, LLP
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 699
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A pharmaceutical preparation containing a complex consisting of type B botulinum neurotoxin and stabilizing proteins, both derived from C. botulinum, admixed with a pharmaceutically acceptable excipient is provided. The preparation is effective for inducing titratable, local, selective muscle denervation in a patient suffering from a disorder characterized by involuntary muscle spasm or contraction.

L7 ANSWER 3 OF 6 USPATFULL
AN 97:12173 USPATFULL
TI Avian antitoxins to clostridium difficle toxin A
IN Williams, James A., Madison, WI, United States
Kink, John A., Madison, WI, United States
Clemens, Christopher M., Madison, WI, United States
Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5601823 970211
AI US 93-161907 931202 (8)
RLI Continuation-in-part of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 3128
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes methods and compositions for treating humans and other animals intoxicated with at least one **Clostridial toxin** by administration of antitoxin. In particular, the antitoxin directed against these toxins is produced in avian species. This avian antitoxin is designed so as to be orally administerable in therapeutic amounts and may be in any form (i.e., as a solid or in aqueous solution).

L7 ANSWER 4 OF 6 USPATFULL
AN 97:9776 USPATFULL
TI Therapy for **clostridial** botulinum **toxin**
IN Carroll, Sean B., Cottage Grove, WI, United States
van Boldrik, Margaret B., Cottage Grove, WI, United States
Clemens, Christopher M., Madison, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5599539 970204
AI US 94-255009 940607 (8)
RLI Continuation of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 92-842709, filed on 26 Feb 1992 which is a continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 10

ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1339
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Treating humans and animals intoxicated with a bacterial toxin by administration of antitoxin. Avian antitoxin in an aqueous solution in therapeutic amount that is orally administrable.

L7 ANSWER 5 OF 6 USPATFULL
AN 96:91828 USPATFULL
TI Method to prevent side-effects and insensitivity to the therapeutic uses of toxins
IN Arnon, Stephen S., 9 Fleetwood Ct., Orinda, CA, United States 94563
PI US 5562907 961008
AI US 94-254238 940606 (8)
RLI Continuation-in-part of Ser. No. US 93-62110, filed on 14 May 1993, now abandoned
PRAI WO 94-US2521 940308
DT Utility
EXNAM Primary Examiner: Scheiner, Toni R.
LREP Morrison & Foerster
CLMN Number of Claims: 16
ECL Exemplary Claim: 16
DRWN No Drawings
LN.CNT 1546
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human-derived or human-compatible antitoxins are administered is an adjunct to therapy with a toxin, such as botulinum toxin or an immunotoxin, or as an adjunct to therapy with a combination of toxins, in order to reduce or prevent endogenous production of antibodies to the toxin(s) or other unwanted side-effects.

L7 ANSWER 6 OF 6 USPATFULL
AN 87:86010 USPATFULL
TI Vaccines based on insoluble supports
IN Wilkins, Tracy D., Blacksburg, VA, United States
Lyerly, David M., Radford, VA, United States
PA Research Corporation, New York, NY, United States (U.S. corporation)
PI US 4713240 871215
AI US 85-719775 850404 (6)
DT Utility
EXNAM Primary Examiner: Kight, John; Assistant Examiner: Draper, Garnette D.
LREP Scully, Scott, Murphy & Presser
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 431
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to vaccine compositions which comprise at least one antigen chemically **linked** to a water-insoluble support combined with a pharmaceutically acceptable carrier. It also relates to a method of stimulating an organism's immune system by administration of these vaccine compositions.

=> s clostridial (5a) neurotoxin?

97 CLOSTRIDIAL
512 NEUROTOXIN?
L8 3 CLOSTRIDIAL (5A) NEUROTOXIN?

=> d bib ab 1-3

L8 ANSWER 1 OF 3 USPATFULL
AN 97:115238 USPATFULL
TI Pharmaceutical composition containing botulinum B complex
IN Johnson, Eric A., Madison, WI, United States
Goodnough, Michael C., Madison, WI, United States
Borodic, Gary E., Canton, MA, United States
PA Associated Synapse Biologics, Cambridge, MA, United States (U.S. corporation)
PI US 5696077 971209
AI US 94-316820 941003 (8)
RLI Continuation of Ser. No. US 93-140328, filed on 20 Oct 1993, now abandoned
DT Utility
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Wai, Thanda
LREP Testa, Hurwitz & Thibeault, LLP
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 699
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A pharmaceutical preparation containing a complex consisting of type B botulinum neurotoxin and stabilizing proteins, both derived from C. botulinum, admixed with a pharmaceutically acceptable excipient is provided. The preparation is effective for inducing titratable, local, selective muscle denervation in a patient suffering from a disorder characterized by involuntary muscle spasm or contraction.

L8 ANSWER 2 OF 3 USPATFULL
AN 97:63883 USPATFULL
TI Cellubrevin homolog
IN Stuart, Susan G., Montara, CA, United States
Hawkins, Phillip R., Mountain View, CA, United States
Seilhamer, Jeffrey J., Los Altos Hills, CA, United States
PA Incyte Pharmaceuticals, Inc., Palo Alto, CA, United States (U.S. corporation)
PI US 5650280 970722
AI US 95-409373 950323 (8)
DT Utility
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Incyte Pharmaceuticals, Inc.; Luther, Barbara J.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides nucleotide and amino acid sequences that identify and encode a novel cellubrevin (cb). The present invention also provides for antisense molecules to the nucleotide sequences which encode cb, expression vectors for the production of purified CB, antibodies capable of binding specifically to CB, hybridization probes or oligonucleotides for the detecting the upregulation of CB encoding nucleotide sequences, genetically engineered host cells for the expression of CB, diagnostic tests for activated, inflamed or diseased cells and/or tissues based on CB-encoding nucleic acid molecules and antibodies capable of binding specifically to CB.

L8 ANSWER 3 OF 3 USPATFULL
AN 96:91828 USPATFULL
TI Method to prevent side-effects and insensitivity to the therapeutic uses of toxins

```
IN      Arnon, Stephen S., 9 Fleetwood Ct., Orinda, CA, United States
        94563
PI      US 5562907  961008
AI      US 94-254238  940606 (8)
RLI     Continuation-in-part of Ser. No. US 93-62110, filed on 14 May
        1993, now abandoned
PRAI    WO 94-US2521  940308
DT      Utility
EXNAM   Primary Examiner: Scheiner, Toni R.
LREP    Morrison & Foerster
CLMN    Number of Claims: 16
ECL     Exemplary Claim: 16
DRWN    No Drawings
LN.CNT 1546
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      Human-derived or human-compatible antitoxins are administered is
        an adjunct to therapy with a toxin, such as botulinum toxin or an
        immunotoxin, or as an adjunct to therapy with a combination of
        toxins, in order to reduce or prevent endogenous production of
        antibodies to the toxin(s) or other unwanted side-effects.

=> s botulinum (5a) toxin? or tetanus (5a) toxin?

           379 BOTULINUM
          7082 TOXIN?
           108 BOTULINUM (5A) TOXIN?
          1341 TETANUS
          7082 TOXIN?
           263 TETANUS (5A) TOXIN?
L9         346 BOTULINUM (5A) TOXIN? OR TETANUS (5A) TOXIN?

=> s l9 and (inactive or modified)

         56599 INACTIVE
        437524 MODIFIED
L10        202 L9 AND (INACTIVE OR MODIFIED)

=> s l10 and (conjugat? or fused or fusion or linked or attach?)

         45443 CONJUGAT?
         67646 FUSED
         39343 FUSION
        126995 LINKED
        855828 ATTACH?
L11        178 L10 AND (CONJUGAT? OR FUSED OR FUSION OR LINKED OR ATTACH?
               )

=> s l11 and (drug? or bioactive or antigen? or inhibitor?)

         62774 DRUG?
          2797 BIOACTIVE
         22817 ANTIGEN?
         79400 INHIBITOR?
L12        177 L11 AND (DRUG? OR BIOACTIVE OR ANTIGEN? OR INHIBITOR?)

=> s l12 and neurotransmitter?

          2769 NEUROTRANSMITTER?
L13          5 L12 AND NEUROTRANSMITTER?

=> d bib ab 1-5


L13  ANSWER 1 OF 5  USPATFULL
AN     97:90887  USPATFULL
```

TI Device for treating gastrointestinal muscle disorders and other smooth muscle dysfunction
IN Pasricha, Pankaj J., Columbia, MD, United States
Kalloo, Anthony N., Glenndale, MD, United States
PA The Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)
PI US 5674205 971007
AI US 95-419933 950411 (8)
RLI Division of Ser. No. US 93-112088, filed on 26 Aug 1993, now patented, Pat. No. US 5437291
DT Utility
EXNAM Primary Examiner: Bockelman, Mark; Assistant Examiner: Smith, Chalin
LREP Banner & Witcoff, Ltd.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 804
AB Direct injection of sphincteric **botulinum toxin** is disclosed as an effective, safe and simple method of treatment for disorders of gastrointestinal muscle or smooth muscles elsewhere in the body, with results that appear to be sustained for several months. Muscle disorders which are suitable for such treatment include achalasia, isolated disorders of the lower esophageal sphincter, gastroparesis, hypertrophic pyloric stenosis, sphincter of Oddi dysfunction, short-segment Hirschsprung's, anal fissure, hemorrhoids, proctalgia fugax, irritable bowel syndrome, disorders of the upper esophageal sphincter, vasospastic disorders, and disorders of uterine and bladder spasm. Devices suitable for delivering this therapy are also disclosed.

L13 ANSWER 2 OF 5 USPATFULL
AN 97:63883 USPATFULL
TI Cellubrevin homolog
IN Stuart, Susan G., Montara, CA, United States
Hawkins, Phillip R., Mountain View, CA, United States
Seilhamer, Jeffrey J., Los Altos Hills, CA, United States
PA Incyte Pharmaceuticals, Inc., Palo Alto, CA, United States (U.S. corporation)
PI US 5650280 970722
AI US 95-409373 950323 (8)
DT Utility
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Incyte Pharmaceuticals, Inc.; Luther, Barbara J.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides nucleotide and amino acid sequences that identify and encode a novel cellubrevin (cb). The present invention also provides for antisense molecules to the nucleotide sequences which encode cb, expression vectors for the production of purified CB, antibodies capable of binding specifically to CB, hybridization probes or oligonucleotides for the detecting the upregulation of CB encoding nucleotide sequences, genetically engineered host cells for the expression of CB, diagnostic tests for activated, inflamed or diseased cells and/or tissues based on CB-encoding nucleic acid molecules and antibodies capable of binding specifically to CB.

L13 ANSWER 3 OF 5 USPATFULL
AN 97:29197 USPATFULL
TI Method for increasing the viability of cells which are

administered to the brain or spinal cord
IN Cherksey, Bruce D., Hoboken, NJ, United States
PA New York University, New York, NY, United States (U.S. corporation)
PI US 5618531 970408
AI US 93-91629 930713 (8)
RLI Continuation of Ser. No. US 92-823654, filed on 23 Jan 1992, now abandoned which is a continuation-in-part of Ser. No. US 90-599802, filed on 19 Oct 1990, now abandoned
DT Utility
EXNAM Primary Examiner: Wityshyn, Michael G.; Assistant Examiner: Dadio, Susan M.
LREP Pennie & Edmonds
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1437
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method for increasing the viability of viable cells which are administered to the brain or spinal cord of a mammalian subject. This method is accomplished by **attaching** the cell to a support matrix so that the cell **attaches** to the matrix surface, and implanting the support matrix with the **attached** cell into the brain or spinal cord. Preferred support matrices are glass or plastic microbeads, either solid or porous, having a diameter from about 90 to about 125 .mu.m. The method employs cells of different types, preferably cells of neural or paraneural origin, such as adrenal chromaffin cells. Also useful are cell lines grown in vitro. Cells not of neural or paraneural origin, such as fibroblasts, may also be used following genetic alteration to express a desired neural product such as a **neurotransmitter** or a neuronal growth factor. The method is used to treat neurological diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, epilepsy, and traumatic brain injury.

L13 ANSWER 4 OF 5 USPATFULL
AN 95:68524 USPATFULL
TI Method for treating gastrointestinal muscle disorders and other smooth muscle dysfunction
IN Pasricha, Pankai J., 5007 Southern Star Ter., Columbia, MD, United States 21044
Kalloo, Anthony N., 10708 Forestgate Pl., Glenndale, MD, United States 20769
PI US 5437291 950801
AI US 93-112088 930826 (8)
DT Utility
EXNAM Primary Examiner: Yasko, John D.; Assistant Examiner: Smith, Chalin
CLMN Number of Claims: 24
ECL Exemplary Claim: 1
DRWN 19 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 765
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Direct injection of sphincteric **botulinum toxin** is disclosed as an effective, safe and simple method of treatment for disorders of gastrointestinal muscle or smooth muscles elsewhere in the body, with results that appear to be sustained for several months. Muscle disorders which are suitable for such treatment include achalasia, isolated disorders of the lower esophageal sphincter, gastroparesis, hypertrophic pyloric stenosis, sphincter of Oddi dysfunction, short-segment Hirschsprung's, anal fissure, hemorrhoids, proctalgia fugax, irritable bowel syndrome, disorders of the upper esophageal sphincter, vasospastic disorders, and disorders of uterine and

bladder spasm. Devices suitable for delivering this therapy are also disclosed.

L13 ANSWER 5 OF 5 USPATFULL
AN 92:53227 USPATFULL
TI Method for the determination and measurements of more than one unknown material in a single surface of a multianalytic assay
IN Fish, Falk, 5 Kashani Street, Tel Aviv, Israel 69499
Herzberg, Max, Moshay Sataria, Rehovot, Israel 73272
Ritterband, Menachem, 25 E. Ben Yehuda Street, Rehovot, Israel 70650
PI US 5126276 920630
AI US 87-113395 871019 (7)
RLI Continuation of Ser. No. US 84-675439, filed on 27 Nov 1984, now abandoned
DT Utility
EXNAM Primary Examiner: Kepplinger, Esther L.; Assistant Examiner: Spiegel, Carol A.
LREP Ostrolenk, Faber, Gerb & Soffen
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 1052
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A solid phase immuno-assay system for assaying at least one analyte, in the form of a solid support having a plurality of receptors bound thereto. At least two of the receptors **conjugate** with the same analyte.

A reaction container comprising a plurality of longitudinally arranged individual compartments, and a longitudinally extending single compartment.

A card for assaying a plurality of samples for the same analyte, having a plurality of receptors for the analyte at different locations on the card.

A method of performing an assay for the same analyte in more than one sample, by providing a receptor for the analyte at more than a single location on a solid substrate; exposing each of the receptors to different samples; and developing each of the receptor locations to indicate the presence of the analyte in each of the samples.

=> d his

(FILE 'HOME' ENTERED AT 11:54:03 ON 15 MAR 1998)

FILE 'USPATFULL' ENTERED AT 11:56:13 ON 15 MAR 1998
E DOLLY JAMES OLIVER/AU
E AOKI KEI ROGER/AU
L1 31 S E2 OR E3
L2 1 S L1 AND TOXIN?
E GARST MICHAEL ELWOOD/AU
L3 40 S E2
L4 1 S L3 AND TOXIN?
E DOLLY JAMES/AU
L5 0 S CLOSTRITDAL (5A) TOXIN?
L6 8 S CLOSTRIDIAL (5A) TOXIN?
L7 6 S L6 AND (CONJUGAT? OR FUSION OR LINK? OR FUSED)
L8 3 S CLOSTRIDIAL (5A) NEUROTOXIN?
L9 346 S BOTULINUM (5A) TOXIN? OR TETANUS (5A) TOXIN?
L10 202 S L9 AND (INACTIVE OR MODIFIED)
L11 178 S L10 AND (CONJUGAT? OR FUSED OR FUSION OR LINKED OR ATTA

L12 177 S L11 AND (DRUG? OR BIOACTIVE OR ANTIGEN? OR INHIBITOR?)
L13 5 S L12 AND NEUROTRANSMITTER?

=> s l12 and synaptobrevin

8 SYNAPTOBREVIN
L14 1 L12 AND SYNAPTOBREVIN

=> d bib ab

L14 ANSWER 1 OF 1 USPATFULL
AN 97:63883 USPATFULL
TI Cellubrevin homolog
IN Stuart, Susan G., Montara, CA, United States
Hawkins, Phillip R., Mountain View, CA, United States
Seilhamer, Jeffrey J., Los Altos Hills, CA, United States
PA Incyte Pharmaceuticals, Inc., Palo Alto, CA, United States (U.S. corporation)
PI US 5650280 970722
AI US 95-409373 950323 (8)
DT Utility
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Incyte Pharmaceuticals, Inc.; Luther, Barbara J.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides nucleotide and amino acid sequences that identify and encode a novel cellubrevin (cb). The present invention also provides for antisense molecules to the nucleotide sequences which encode cb, expression vectors for the production of purified CB, antibodies capable of binding specifically to CB, hybridization probes or oligonucleotides for the detecting the upregulation of CB encoding nucleotide sequences, genetically engineered host cells for the expression of CB, diagnostic tests for activated, inflamed or diseased cells and/or tissues based on CB-encoding nucleic acid molecules and antibodies capable of binding specifically to CB.

=> d kwic

L14 ANSWER 1 OF 1 USPATFULL
SUMM . . . to RA, and several models have been developed. These models have in common the generation of an immune response against **antigens** present in the rheumatoid joint. There is some evidence that the initial response may have been to viral **antigens**. In this scenario, ongoing immune response may be due to low levels of **antigen** persisting in the joint or a crossreaction to joint structures. Alternatively, the immunologic activity observed in RA may occur in. . .
SUMM . . . which recognizes autologous Fc. These polyclonal antibodies may be induced by any number of sources with the subsequent production of **antigen** as discussed above.
SUMM Cellubrevins are homologues of synaptobrevins, synaptic vesicle-associated membrane proteins (VAMPs). **Synaptobrevin** was first discovered in rat brain (Baumert et al (1989) Embo J 8:379-84) and initially thought to be limited to neuronal cells. **Synaptobrevin** is an integral membrane protein of 18 kDA (Ralston E. et al (1994) J Biol Chem 269:15403-6) involved in the. . . endocytotic process may be blocked by the highly specific action of clostridial neurotoxins which prevents neurotransmitter release by cleaving the

synaptobrevin molecule. Synaptobrevins are now known to occur and function in the receptor-mediated endocytotic pathways of many non-neuronal cell types.

SUMM As mentioned for **synaptobrevin** above, cellubrevins are sensitive to selective proteolysis by metalloendoproteases such as the zinc endoprotease which comprises the light chain of **tetanus toxin**. Experiments have shown that endosome **fusion** may continue even after specific cellubrevin cleavage through temperature- and ATP-dependent docking and **fusion** processes involving N-ethylmaleimide-sensitive **fusion** proteins (NSF) and small, soluble **attachment** proteins (SNAP).

SUMM . . . this novel homolog (and associated VAMPs) with docking proteins such as syntaxin and SNAPs of the plasmalemma or the core **fusion** proteins such as NSF and the synaptotagmins (Bark I. C. and Wilson M. C. (1994) Proc Natl Acad Sci 91:4621-4624). . .

DRWD FIG. 3 shows amino acid alignment of CB with **synaptobrevin** (S63830) SEQ ID NO:3 and cellubrevin (X76199) SEQ ID NO: 4. Alignments shown were produced using the multisequence alignment program. . .

DETD "Derivative" refers to CBs chemically **modified** by such techniques as ubiquitination, labeling (e.g., with radionuclides, various enzymes, etc.), pegylation (derivatization with polyethylene glycol), and insertion or. . .

DETD . . . contain the entire aa sequence of a small naturally occurring molecules like CB. Short stretches of CB aa may be **fused** with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule.

DETD Antibodies specific for CB may be produced by inoculation of an appropriate animal with the polypeptide or an **antigenic** fragment. An antibody is specific for CB if it is produced against an epitope of the polypeptide and binds to. . .

DETD An additional embodiment of the subject invention is the use of CB specific antibodies, receptors or the like as **bioactive** agents to treat viral or other infections, traumatic tissue damage, hereditary diseases such as arthritis or asthma, invasive leukemias and. . .

DETD **Bioactive** compositions comprising agonists, antagonists, or receptors of CB may be administered in a suitable therapeutic dose determined by any of. . . studies on mammalian species to determine maximum tolerable dose and on normal human subjects to determine safe dosage. Additionally, the **bioactive** agent may be complexed with a variety of well established compounds or compositions which enhance stability or pharmacological properties such as half-life. It is contemplated that a therapeutic, **bioactive** composition may be delivered by intravenous infusion into the bloodstream or any other effective means which could be used for. . .

DETD . . . use of a plasmid system for easy insert characterization, sequencing, site-directed mutagenesis, the creation of unidirectional deletions and expression of **fusion** polypeptides. Subsequently, the custom-constructed library phage particles were infected into E. coli host strain XL1-Blue.RTM. (Stratagene). The high transformation efficiency. . .

DETD . . . sequences of CB. FIG. 2 shows the hydrophobicity plot for CB. FIG. 3 shows the amino acid alignment of CB **synaptobrevin** (S63830) SEQ ID NO:3 and cellubrevin (X76199) SEQ ID NO:4.

DETD Induction of the isolated, transfected bacterial strain with IPTG using standard methods will produce a **fusion** protein corresponding to the first seven residues of .beta.-galactosidase, about 15 residues of "linker", and the peptide encoded within the. . .

DETD . . . them, either covalently or noncovalently, with a

substance which provides for a detectable signal. A wide variety of labels and **conjugation** techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, **inhibitors**, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Pat. Nos.. . .

DETD . . . or membrane-bound CB, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-**linked** immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to. . .

DETD . . . prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently **attached** to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin. . .

DETD XII. **Drug** Screening

DETD . . . is particularly useful for screening therapeutic compounds by using CB or binding fragments thereof in any of a variety of **drug** screening techniques. The polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of **drug** screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. **Drugs** are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be. . .

DETD Thus, the present invention provides methods of screening for **drugs** or any other agents which can affect vesicular trafficking. These methods comprise contacting such an agent with CB polypeptide or. . .

DETD Another technique for **drug** screening provides high throughput screening for compounds having suitable binding affinity to the CB polypeptides and is described in detail. . . methods well known in the art. Purified CB can also be coated directly onto plates for use in the aforementioned **drug** screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

DETD This invention also contemplates the use of competitive **drug** screening assays in which neutralizing antibodies capable of binding CB specifically compete with a test compound for binding to CB. . . In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more **antigenic** determinants with CB.

DETD XIII. Rational **Drug** Design

DETD The goal of rational **drug** design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, e.g., agonists, antagonists, or **inhibitors**. Any of these examples can be used to fashion **drugs** which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a. . .

DETD In one approach, the three-dimensional structure of a protein of interest, or of a protein-**inhibitor** complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both. . . by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design efficient **inhibitors**. Useful examples of rational **drug** design may include molecules which have improved activity or

stability as shown by Braxton S. and Wells J. A. (1992 Biochemistry 31:7796-7801) or which act as **inhibitors**, agonists, or antagonists of native peptides as shown by Athauda S. B. et al (1993 J Biochem 113:742-746), incorporated herein. . .

DETD . . . as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent **drug** design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional,. . .

DETD The inventive purified CB is a research tool for identification, characterization and purification of receptors, docking and **fusion** proteins. Radioactive labels may be incorporated into CB by various methods known in the art and used to capture either. . .

DETD XV. Use and Administration of Antibodies, **Inhibitors**, Receptors or

DETD Antibodies, **inhibitors**, receptors or antagonists of CB (or other treatments to limit vesicular trafficking, TCB), can provide different effects when administered therapeutically.. . . 5 to 8, more preferably 6 to 8, although the pH may vary according to the characteristics of the antibody, **inhibitor**, or antagonist being formulated and the condition to be treated. Characteristics of TCBs include solubility of the molecule, half-life and **antigenicity** /immunogenicity; these and other characteristics may aid in defining an effective carrier. Native human proteins are preferred as TCBs, but organic or synthetic molecules resulting from **drug** screens may be equally effective in particular situations.

DETD . . . may be taken into account include disease state (e.g. severity) of the patient, age, weight, gender, diet, time of administration, **drug** combination, reaction sensitivities, and tolerance/response to therapy. Long acting TCB formulations might be administered every 3 to 4 days, every. . .

DETD . . . and consists of a therapeutic peptide. Whereas the peptide is protected within the vesicle during delivery; at the time of **fusion**, it is exposed and effectively becomes part of the intracellular plasmalemma. The exposed peptide either carries out its function while. . .

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=> s l12 and (botulism or tetanus)

           112 BOTULISM
          1341 TETANUS
L15        164 L12 AND (BOTULISM OR TETANUS)

=> s l15 and botulism

           112 BOTULISM
L16         10 L15 AND BOTULISM

=> d bib ab 1-10


L16  ANSWER 1 OF 10  USPATFULL
AN     1998:17427  USPATFULL
TI     Clostridial toxin disease therapy
IN     Carroll, Sean B., Cottage Grove, WI, United States
       van Boldrik, Margaret B., Cottage Grove, WI, United States
       Clemens, Christopher M., Madison, WI, United States
PA     Ophidian Pharmaceuticals Inc., Madison, WI, United States (U.S.
       corporation)
PI     US 5719267  980217
AI     US 95-457890  950601 (8)
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RLI Division of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll,LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1310
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Treating humans and animals intoxicated with a bacterial toxin by administration of antitoxin. Avian antitoxin in an aqueous solution in therapeutic amount that is orally administrable.

L16 ANSWER 2 OF 10 USPATFULL
AN 97:12173 USPATFULL
TI Avian antitoxins to clostridium difficle toxin A
IN Williams, James A., Madison, WI, United States
Kink, John A., Madison, WI, United States
Clemens, Christopher M., Madison, WI, United States
Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5601823 970211
AI US 93-161907 931202 (8)
RLI Continuation-in-part of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 3128
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention includes methods and compositions for treating humans and other animals intoxicated with at least one Clostridial toxin by administration of antitoxin. In particular, the antitoxin directed against these toxins is produced in avian species. This avian antitoxin is designed so as to be orally administerable in therapeutic amounts and may be in any form (i.e., as a solid or in aqueous solution).

L16 ANSWER 3 OF 10 USPATFULL
AN 97:9776 USPATFULL
TI Therapy for clostridial **botulinum toxin**
IN Carroll, Sean B., Cottage Grove, WI, United States
van Boldrik, Margaret B., Cottage Grove, WI, United States
Clemens, Christopher M., Madison, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5599539 970204
AI US 94-255009 940607 (8)
RLI Continuation of Ser. No. US 92-985321, filed on 4 Dec 1992 which is a continuation-in-part of Ser. No. US 92-842709, filed on 26 Feb 1992 which is a continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Medlen & Carroll, LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 2 Drawing Figure(s); 2 Drawing Page(s)

LN.CNT 1339
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Treating humans and animals intoxicated with a bacterial toxin by administration of antitoxin. Avian antitoxin in an aqueous solution in therapeutic amount that is orally administrable.

L16 ANSWER 4 OF 10 USPATFULL
AN 96:116111 USPATFULL
TI Dual carrier immunogenic construct
IN Mond, James J., Potomac, MD, United States
Lees, Andrew, Baltimore, MD, United States
PA Henry Jackson Foundation, Rockville, MD, United States (U.S. corporation)
PI US 5585100 961217
AI US 95-402565 950313 (8)
RLI Continuation of Ser. No. US 93-126017, filed on 24 Sep 1993, now abandoned which is a continuation of Ser. No. US 92-834067, filed on 11 Feb 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Krsek-Staples, Julie
LREP Finnegan, Henderson, Farabow, Garrett and Dunner, L.L.P.
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 14 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 1143
AB A dual carrier immunogenic construct comprised of at least one primary carrier comprising large molecular weight molecule of greater than a 70 KD molecular weight and at least one secondary carrier comprising a T-dependent **antigen** **conjugated** to a primary carrier. The dual carrier immunogenic construct may further comprise moieties such as haptens and **antigens**. Such immunogenic constructs are suitable for use in the diagnosis, treatment, and prevention of diseases.

L16 ANSWER 5 OF 10 USPATFULL
AN 96:94465 USPATFULL
TI Enhancer sequence for modulating expression in epithelial cells
IN Kufe, Donald, Wellesley, MA, United States
Abe, Miyako, Boston, MA, United States
PA Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)
PI US 5565334 961015
AI US 94-324465 941017 (8)
RLI Continuation of Ser. No. US 92-999742, filed on 31 Dec 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Elliott, George C.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 867
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated DNA encompassing the DF3 enhancer as well as a sequence encoding a heterologous polypeptide provides epithelial tissue-selective gene expression of the heterologous polypeptide, useful in methods of therapy.

L16 ANSWER 6 OF 10 USPATFULL
AN 96:91828 USPATFULL
TI Method to prevent side-effects and insensitivity to the therapeutic uses of toxins
IN Arnon, Stephen S., 9 Fleetwood Ct., Orinda, CA, United States

94563
PI US 5562907 961008
AI US 94-254238 940606 (8)
RLI Continuation-in-part of Ser. No. US 93-62110, filed on 14 May 1993, now abandoned
PRAI WO 94-US2521 940308
DT Utility
EXNAM Primary Examiner: Scheiner, Toni R.
LREP Morrison & Foerster
CLMN Number of Claims: 16
ECL Exemplary Claim: 16
DRWN No Drawings
LN.CNT 1546
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Human-derived or human-compatible antitoxins are administered is an adjunct to therapy with a **toxin**, such as **botulinum toxin** or an immunotoxin, or as an adjunct to therapy with a combination of toxins, in order to reduce or prevent endogenous production of antibodies to the toxin(s) or other unwanted side-effects.

L16 ANSWER 7 OF 10 USPATFULL
AN 96:36543 USPATFULL
TI Pharmaceutical composition of botulinum neurotoxin and method of preparation
IN Johnson, Eric A., Madison, WI, United States
Goodnough, Michael C., Madison, WI, United States
PA Wisconsin Alumni Research Foundation, Madison, WI, United States (U.S. corporation)
PI US 5512547 960430
AI US 94-322624 941013 (8)
DT Utility
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Mohamed, Abdel A.
LREP Quarles & Brady
CLMN Number of Claims: 3
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 365
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Pharmaceutical compositions of botulinum neurotoxin containing higher specific toxicity and increased stability at higher temperatures than currently available preparations.

L16 ANSWER 8 OF 10 USPATFULL
AN 95:75887 USPATFULL
TI Immobilization of Crotalus atrox and Crotalus durissus terrificus whole venoms on aldehyde-activated agarose
IN Carroll, Sean B., 3066 Streb Way, Cottage Grove, WI, United States 53527
PI US 5443976 950822
AI US 94-275304 940714 (8)
RLI Continuation of Ser. No. US 92-983668, filed on 1 Dec 1992, now abandoned which is a division of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Naff, David M.
LREP Haverstock, Medlen & Carroll
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 3798
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Antivenoms to snake, spider, scorpion and jelly fish venoms are produced for the treatment of humans and animals, and for

analytical use. The antivenom is purified with an **antigen** matrix containing a single whole venom or a plurality of whole venoms covalently **attached** to an insoluble support such as aldehyde-activated agarose. Preferably, the whole venoms forming the plurality of whole venoms are selected from the four whole venoms from C. atrox, B. atrox, C. adamanteus and C. durissus terrificus. A combination of immobilized C. atrox and C. durissus terrificus whole venoms can substantially purify antivenom reactive with all four venoms. The antivenom can be horse or avian such as chicken antivenom.

L16 ANSWER 9 OF 10 USPATFULL
AN 94:73408 USPATFULL
TI Methods for making and purifying antivenoms
IN Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5340923 940823
AI US 92-977583 921117 (7)
DCD 20060323
RLI Continuation-in-part of Ser. No. US 89-429791, filed on 31 Oct 1989, now patented, Pat. No. US 5196193
DT Utility
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Eisenschenk, F. C.
LREP Haverstock, Medlen & Carroll
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 3845
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Antivenoms suitable for treatment of humans and animals as well as for analytical use. A method wherein individual venoms are used to immunize and the resulting antivenoms are, thereafter, purified individually prior to mixing. Immunization is performed in a mammalian or avian host species.

L16 ANSWER 10 OF 10 USPATFULL
AN 93:22480 USPATFULL
TI Antivenoms and methods for making antivenoms
IN Carroll, Sean B., Cottage Grove, WI, United States
PA Ophidian Pharmaceuticals, Inc., Madison, WI, United States (U.S. corporation)
PI US 5196193 930323
AI US 89-429791 891031 (7)
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Baker, R.
LREP Haverstock, Medlen & Carroll
CLMN Number of Claims: 31
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 3868
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The production of antivenoms in non-mammals and improvements in the effectiveness of both non-mammalian antivenoms and mammalian antivenoms so that they are more suitable for treatment of humans and animals as well as for analytical use.

=> s inactive (10a) clostridial (5a) neurotoxin

56599 INACTIVE
97 CLOSTRIDIAL
323 NEUROTOXIN
L17 0 INACTIVE (10A) CLOSTRIDIAL (5A) NEUROTOXIN

```
=> s inactive (10a) botulin (5a) neurotoxin

          56599 INACTIVE
             17 BOTULIN
            323 NEUROTOXIN
L18           0 INACTIVE (10A) BOTULIN (5A) NEUROTOXIN

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=> s cellubrevin

L1 271 CELLUBREVIN

=> s l1 and (tetanus or botulinum)

L2 108 L1 AND (TETANUS OR BOTULINUM)

=> s l2 and neurotransmit?

L3 18 L2 AND NEUROTRANSMIT?

=> dup rem l3

PROCESSING COMPLETED FOR L3
L4 8 DUP REM L3 (10 DUPLICATES REMOVED)

=> d bib ab 1-8

L4 ANSWER 1 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95207029 EMBASE
TI VAMP-2 and **cellubrevin** are expressed in pancreatic .beta.-cells and are essential for Ca2+ - but not for GTP.gamma.S-induced insulin secretion.
AU Regazzi R.; Wollheim C.B.; Lang J.; Theler J.-M.; Rossetto O.; Montecucco C.; Sadoul K.; Weller U.; Palmer M.; Thorens B.
CS Division of Clinical Biochemistry, Department of Medicine, University of Geneva, Geneva 1211, Switzerland
SO EMBO Journal, (1995) 14/12 (2723-2730).
ISSN: 0261-4189 CODEN: EMJODG
CY United Kingdom
DT Journal
FS 002 Physiology
022 Human Genetics
029 Clinical Biochemistry
LA English
SL English
AB VAMP proteins are important components of the machinery controlling docking and/or fusion of secretory vesicles with their target membrane. We investigated the expression of VAMP proteins in pancreatic .beta.-cells and their implication in the exocytosis of insulin. cDNA cloning revealed that VAMP-2 and **cellubrevin** , but not VAMP-1, are expressed in rat pancreatic islets and that their sequence is identical to that isolated from rat brain. Pancreatic .beta.-cells contain secretory granules that store and secrete insulin as well as synaptic-like microvesicles carrying .gamma.-aminobutyric acid. After subcellular fractionation on continuous sucrose gradients, VAMP-2 and **cellubrevin** were

found to be associated with both types of secretory vesicle. The association of VAMP-2 with insulin-containing granules was confirmed by confocal microscopy of primary cultures of rat pancreatic .beta.-cells. Pretreatment of streptolysin-O permeabilized insulin-secreting cells with **tetanus** and **botulinum** B neurotoxins selectively cleaved VAMP-2 and **cellubrevin** and abolished Ca2+ induced insulin release (IC50 .apprx. 15 nM). By contrast, the pretreatment with **tetanus** and **botulinum** B neurotoxins did not prevent GTP.gamma.S-stimulated insulin secretion. Taken together, our results show that pancreatic .beta.-cells express VAMP-2 and **cellubrevin** and that one or both of these proteins selectively control Ca2+-mediated insulin secretion.

L4 ANSWER 2 OF 8 BIOSIS COPYRIGHT 1998 BIOSIS
AN 96:112049 BIOSIS
DN 98684184
TI Expression of synaptobrevin II, **cellubrevin** and syntaxin but not SNAP-25 in cultured astrocytes.
AU Parpura V; Fang Yu; Basarsky T; Jahn R; Haydon P G
CS Lab. Cellular Signaling, Dep. Zool. Genetics, 339 Science II, Iowa State Univ., Ames, IA 50011, USA
SO FEBS Letters 377 (3). 1995. 489-492. ISSN: 0014-5793
LA English
AB Astrocytes, a sub-type of glial cell in the central nervous system, can release the excitatory transmitters glutamate and aspartate in response to elevated levels of internal calcium. To investigate potential release mechanisms that may be present in these cells we have determined whether protein components of the neuronal secretory apparatus are expressed in astrocytes. Western blots, immunocytochemistry and RT PCR demonstrate that astrocytes express **cellubrevin**, synaptobrevin II and syntaxin, proteins known to form a macromolecular fusion complex. However, SNAP-25 which is another neuronal protein of the fusion complex, was not detected. Astrocyte **cellubrevin** and synaptobrevin II were also shown to be sensitive to the proteolytic activity of **tetanus** toxin. Together these data indicate that astrocytes express some proteins that are known to form a fusion complex indicating that regulated exocytosis might mediate calcium-regulated transmitter release from these cells.

L4 ANSWER 3 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 96201698 EMBASE
TI Molecular mechanisms in synaptic vesicle endocytosis.
AU Bauerfeind R.; David C.; Galli T.; McPherson P.S.; Takei K.; De Camilli P.
CS Department of Cell Biology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510, United States
SO Cold Spring Harbor Symposia on Quantitative Biology, (1995) 60/- (397-404).
ISSN: 0091-7451 CODEN: CSHSAZ
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English

L4 ANSWER 4 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 1
AN 94327967 EMBASE
TI Cytotoxic effects of a chimeric protein consisting of **tetanus** toxin light chain and anthrax toxin lethal factor in non-neuronal cells.
AU Arora N.; Williamson L.C.; Leppla S.H.; Halpern J.L.
CS Bldg. 29, 8800 Rockville Pike, Bethesda, MD 20892, United States
SO J. BIOL. CHEM., (1994) 269/42 (26165-26171).

ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB The light chain of **tetanus** toxin is a zinc endoprotease that inhibits **neurotransmitter** release by selective proteolysis of the synaptic vesicle- associated protein synaptobrevin/vesicle-associated membrane protein. **Cellubrevin** is a homologue of synaptobrevin that is found in most cell types and is also a substrate for **tetanus** toxin. The lack of receptors for **tetanus** toxin on most cell types has made studies of **tetanus** toxin action in non- neuronal cells difficult. To characterize **tetanus** toxin effects in non- neuronal cells, a fusion protein consisting of the 254 amino-terminal amino acids of lethal factor (LF) of anthrax toxin and **tetanus** toxin light chain (LC) was prepared. This protein (LF-LC) inhibited evoked glycine release from primary spinal cord neurons at concentrations between 1.0 and 100 ng/ml. LF- LC was cytotoxic to RAW 264.7, ANA-1 cells (mouse macrophage cell lines), and Chinese hamster ovary cells in a dose-dependent manner. These effects required the presence of protective antigen, the receptor binding component of anthrax toxin. In contrast, LF-LC was not cytotoxic to RBL-2H3, Vero, or mouse hybridoma cell lines. Mutagenesis of conserved amino acids (His237 and Glu234) in the zinc-binding motif of LC resulted in fusion proteins having no biological activity. LF-LC did not inhibit regulated secretion of serotonin in RBL-2H3 cells or constitutive secretion in any non-neuronal cell lines as measured in several different assays. We suggest that the cytotoxic effects of LF-LC result from inhibition of a specific intracellular membrane fusion event mediated by **cellubrevin**.

L4 ANSWER 5 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 2
AN 94166806 EMBASE
TI **Tetanus** toxin-mediated cleavage of **cellubrevin** impairs exocytosis of transferrin receptor-containing vesicles in CHO cells.
AU Galli T.; Chilcote T.; Mundigl O.; Binz T.; Niemann H.; De Camilli P.
CS Department of Cell Biology, Howard Hughes Medical Institute, Yale University School of Medicine, 295 Congress Avenue, New Haven, CT 06510, United States
SO J. CELL BIOL., (1994) 125/5 (1015-1024).
ISSN: 0021-9525 CODEN: JCLBA3
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB **Cellubrevin** is a member of the synaptobrevin/VAMP family of SNAREs, which has a broad tissue distribution. In fibroblastic cells it is concentrated in the vesicles which recycle transferrin receptors but its role in membrane trafficking and fusion remains to be demonstrated. **Cellubrevin**, like the synaptic vesicle proteins synaptobrevins I and II, can be cleaved by **tetanus** toxin, a metallo-endoprotease which blocks **neurotransmitter** release. However, nonneuronal cells are unaffected by the toxin due to lack of cell surface receptors for its heavy chain. To determine whether **cellubrevin** cleavage impairs exocytosis of recycling vesicles, we tested the effect of **tetanus** toxin light chain on the release of preinternalized transferrin from streptolysin-O-perforated CHO cells. The release was found to be

temperature and ATP dependent as well as NEM sensitive. Addition of **tetanus** toxin light chain, but not of a proteolytically inactive form of the toxin, resulted in a partial inhibition of transferrin release which correlated with the toxin- mediated cleavage of **cellubrevin**. The residual release of transferrin occurring after complete **cellubrevin** degradation was still ATP dependent. Our results indicate that **cellubrevin** plays an important role in the constitutive exocytosis of vesicles which recycle plasmalemma receptors. The incomplete inhibition of transferrin release produced by the toxin suggests the existence of a **cellubrevin**-independent exocytotic mechanism, which may involve **tetanus** toxin-insensitive proteins of the synaptobrevin/VAMP family.

L4 ANSWER 6 OF 8 CAPLUS COPYRIGHT 1998 ACS
AN 1994:291492 CAPLUS
DN 120:291492
TI Inhibition of **neurotransmitter** release by **tetanus** and **botulinum** neurotoxins
AU Mochida, Sumiko
CS Dep. Physiol., Tokyo Med. Coll., Tokyo, 160, Japan
SO Seikagaku (1994), 66(3), 254-9
CODEN: SEIKAQ; ISSN: 0037-1017
DT Journal; General Review
LA Japanese
AB A review with 16 refs. on double-stranded structures, functions of each fragment, cloning of genes, identification of active sites, and functions as proteases in nerve ending of neurotoxins produced by Clostridium tetani and C. **botulinum**. Target mol. (e.g. VAMP/synaptobrevin, **cellubrevin**, SNAP-25, and syntaxin) of the neurotoxins are described.

L4 ANSWER 7 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 3
AN 93273780 EMBASE
TI Cleavage of **cellubrevin** by **tetanus** toxin does not affect fusion of early endosomes.
AU Link E.; McMahon H.; Von Mollard G.F.; Yamasaki S.; Niemann H.; Sudhof T.C.; Jahn R.
CS Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University Medical School, P.O. Box 9812, New Haven, CT 06536, United States
SO J. BIOL. CHEM., (1993) 268/25 (18423-18426).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB **Tetanus** toxin is a potent inhibitor of **neurotransmitter** release, which acts as an intracellular metalloendoprotease that selectively cleaves synaptobrevin, a major membrane protein of synaptic vesicles. Recently, synaptobrevin has been found to form an ATP-dependent complex with N-ethylmaleimide-sensitive fusion protein (NSF) and soluble NSF attachment protein, which are known to function in endosome fusion. Furthermore, a highly homologous isoform of synaptobrevin, named **cellubrevin**, was identified that is expressed in virtually all tissues in the endocytic pathway and is cleaved by **tetanus** toxin light chain in vitro, suggesting that **cellubrevin** may have a general function in intracellular fusion events. In the present study, we have analyzed whether cleavage of **cellubrevin** by **tetanus** toxin influences the ATP-dependent, N-ethylmaleimide-sensitive fusion of early endosomes in vitro. Our results show that endosome fusion is not affected by **tetanus** toxin although **cellubrevin**

is almost completely proteolyzed, suggesting that the function of NSF in endosome fusion does not involve **cellubrevin**.

L4 ANSWER 8 OF 8 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 4
AN 93265291 EMBASE
TI **Cellubrevin** is a ubiquitous **tetanus**-toxin substrate homologous to a putative synaptic vesicle fusion protein.
AU McMahon H.T.; Ushkaryov Y.A.; Edelmann L.; Link E.; Binz T.; Niemann H.; Jahn R.; Sudhof T.C.
CS Department of Molecular Genetics, Howard Hughes Medical Institute, Texas University SW Medical Center, Dallas, TX 75235, United States
SO NATURE, (1993) 364/6435 (346-349).
ISSN: 0028-0836 CODEN: NATUAS
CY United Kingdom
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB **TETANUS** toxin inhibits **neurotransmitter** release by selectively blocking fusion of synaptic vesicles. Recently **tetanus** toxin was shown to proteolytically degrade synaptobrevin II (also named VAMP-2), a synaptic vesicle-specific protein, in vitro and in nerve terminals. As targets of **tetanus** toxin, synaptobrevins probably function in the exocytotic fusion of synaptic vesicles. Here we describe a new synaptobrevin homologue, **cellubrevin**, that is present in all cells and tissues tested and demonstrate that it is a membrane trafficking protein of a constitutively recycling pathway. Like synaptobrevin II, **cellubrevin** is proteolysed by **tetanus** toxin light chain in vitro and after transfection. Our results suggest that constitutive and regulated vesicular pathways use homologous proteins for membrane trafficking, probably for membrane fusion at the plasma membrane, indicating a greater mechanistic and evolutionary similarity between these pathways than previously thought.

```
=> e dolly james oliver/au

E1          106     DOLLY J OLIVER/AU
E2            3     DOLLY J P/AU
E3            3 --> DOLLY JAMES OLIVER/AU
E4            1     DOLLY JASMINA CAMACHO CORREDOR/AU
E5            1     DOLLY JOHN PATRICK/AU
E6            1     DOLLY M C/AU
E7            1     DOLLY MARTHA R/AU
E8            2     DOLLY MARTIN C/AU
E9           16     DOLLY O/AU
E10           6     DOLLY O J/AU
E11           4     DOLLY OLIVER/AU
E12           2     DOLLY OLIVER J/AU

=> s e1 or e3

L5          109 "DOLLY J OLIVER"/AU OR "DOLLY JAMES OLIVER"/AU

=> s l5 and toxin

L6           62 L5 AND TOXIN

=> dup rem l6

PROCESSING COMPLETED FOR L6
L7           62 DUP REM L6 (0 DUPLICATES REMOVED)

=> s l7 and (clostrid? or botulin? or tetanus)
```

L8 40 L7 AND (CLOSTRID? OR BOTULIN? OR TETANUS)

=> s l8 and vamp

L9 4 L8 AND VAMP

=> d bib ab 1-4

L9 ANSWER 1 OF 4 CAPLUS COPYRIGHT 1998 ACS
AN 1995:220501 CAPLUS
DN 122:3157
TI Differences in the Protease Activities of **Tetanus** and **Botulinum** B Toxins Revealed by the Cleavage of Vesicle-Associated Membrane Protein and Various Sized Fragments
AU Foran, Patrick; Shone, Clifford C.; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1994), 33(51), 15365-74
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB **Botulinum** neurotoxin serotype B (BoNT/B) and **tetanus toxin** (TeTx) block neuroexocytosis through selective endoproteolysis of vesicle-assocd. membrane protein (**VAMP**). The enzymol. properties of both toxins were compared for the first time in their cleavage of **VAMP** and various sized fragments using a sensitive chromatog. assay. The optimal substrate sizes for the zinc-dependent protease activities of the light chains of TeTx and BoNT/B were established using synthetic peptides corresponding to the hydrophilic core of **VAMP** (30-62 amino acids in length). TeTx was found to selectively cleave the largest peptide at a single site, Gln76-Phe77. It exhibited the most demanding specificity, requiring the entire hydrophilic domain (a 62-mer) for notable hydrolysis, whereas BoNT/B efficiently cleaved the much smaller 40-mer. Thus, an unusually long N-terminal sequence of 44 amino acids upstream of the scissile bond is required for the selective hydrolysis of **VAMP** by TeTx. Using the largest peptide, BoNT/B and TeTx exhibited .apprx.50% and 35%, resp., of the activities shown toward intact **VAMP**, detergent solubilized from synaptic vesicles. Given the large size of the smallest substrates, it is possible that these neurotoxins recognize and require a three-dimensional structure. Although both toxins were inactivated by divalent metal chelators, neither was antagonized by phosphoramidon or ASQFETS (a substrate-related peptide that spans the cleavage site), and TeTx was only feebly inhibited by captopril; also, they were distinguishable in their relative activities at different pHs, temps., and ionic strengths. These collective findings are important in the design of effective inhibitors for both toxins, as well as in raising the possibility that TeTx and BoNT/B interact somewhat differently with **VAMP** .

L9 ANSWER 2 OF 4 CAPLUS COPYRIGHT 1998 ACS
AN 1994:570660 CAPLUS
DN 121:170660
TI Probing the process of transmitter release with **botulinum** and **tetanus** neurotoxins
AU **Dolly, J. Oliver**; Paiva, Anton de; Foran, Patrick; Lawrence, Gary; Daniels-Holgate, Phillipa; Ashton, Anthony C.
CS Department Biochemistry, Imperial College, London, SW7 2AZ, UK
SO Semin. Neurosci. (1994), 6(3), 149-58
CODEN: SNEUEZ; ISSN: 1044-5765
DT Journal; General Review

LA English
AB A review, with 54 refs., on **botulinum** neurotoxin (BoNT) and **tetanus toxin** (TeTx), as uniquely specific inhibitors of neuro-exocytosis, which have extended the functional components identifiable in the nervous system with neurotoxins. Resp. actions of these **clostridial** proteins on cholinergic and inhibitory nerve terminals arise from binding via their heavy chains to distinct ecto-acceptors; this is followed by endocytic translocation to the cytosol where zinc-dependent protease activities of their light chains selectively cleave **VAMP**, vesicle-assocd. membrane protein (TeTx, BoNT/B, D, F), and the plasma membrane proteins SNAP-25 (BoNT/A, E) and syntaxin (BoNT/C1)-ubiquitous components essential for the release of, apparently, all neurotransmitters. Pharmacol. differences in the toxins' effects reflect either distinct targets or cleavage sites, subtle features exploitable in deciphering the precise role served by each of these membrane proteins, in exocytosis from neuronal and neuro-endocrine cells.

L9 ANSWER 3 OF 4 CAPLUS COPYRIGHT 1998 ACS
AN 1994:452037 CAPLUS
DN 121:52037
TI **Botulinum** A and the light chain of **tetanus** toxins inhibit distinct stages of Mg . ATP-dependent catecholamine exocytosis from permeabilized chromaffin cells
AU Lawrence, Gary W.; Weller, Ulrich; **Dolly, J. Oliver**
CS Biochem. Dep., Imperial Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO Eur. J. Biochem. (1994), 222(2), 325-33
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB Susceptibilities of Mg.ATP-independent and Mg.ATP-requiring components of catecholamine secretion from digitonin-permeabilized chromaffin cells to inhibition by **Clostridial botulinum** type A and **tetanus** toxins were investigated. These toxins are Zn2+-dependent proteases which specifically cleave the 25-kDa synaptosomal-assocd. protein (SNAP-25) and vesicle-assocd. membrane protein (**VAMP**) II, resp. When applied to permeabilized chromaffin cells they rapidly inhibited secretion in the presence of Mg.ATP but the catecholamine released in the absence of Mg.ATP, thought to represent fusion of primed granules, was not perturbed. The toxins can exert their effects per se in the absence of the nucleotide complex; therefore, Mg.ATP-requiring steps of secretion are implicated as roles for their targets. Primed release was lost rapidly after permeabilization of the cell but could be maintained by including Mg.ATP during the incubation before stimulating release with Ca2+. This ability of Mg.ATP to maintain primed release was only partially inhibited by **botulinum** neurotoxin A whereas it was abolished by **tetanus toxin**, consistent with the distinct substrates for these toxins. This study reveals a component of release within which these proteins are either resistant to cleavage by these toxins or in such a position that degrdn. can no longer prevent granule fusion. Differences in the steps of release at which these toxins can affect inhibition are also revealed.

L9 ANSWER 4 OF 4 CAPLUS COPYRIGHT 1998 ACS
AN 1994:317560 CAPLUS
DN 120:317560
TI A Single Mutation in the Recombinant Light Chain of **Tetanus Toxin** Abolishes Its Proteolytic Activity and Removes the Toxicity Seen after Reconstitution with Native Heavy Chain
AU Li, Yan; Foran, Patrick; Fairweather, Neil F.; de Paiva, Anton;

Weller, Ulrich; Dougan, Gordon; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1994), 33(22), 7014-20
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB Specific proteolysis by the **tetanus toxin** light chain of a vesicle-assocd. membrane protein (**VAMP**) involved in exocytosis is thought to underlie its intracellular blockade of neurotransmitter release. To substantiate this mechanism, recombinant light chain was expressed as a maltose binding protein-light chain fusion product in Escherichia coli. After purifn. by affinity chromatog. and cleavage with factor Xa, the resultant light chain was isolated and its identity confirmed by Western blotting and N-terminal sequencing. It exhibited activity similar to that of the native light chain in proteolyzing its target in isolated bovine small synaptic vesicles and in hydrolyzing a 62-residue synthetic polypeptide spanning the cleavage site of the substrate. The importance of Glu234 in the catalytic activity of the light chain, possibly analogous to Glu143 of thermolysin, was examd. using site-directed mutagenesis. Changing Glu234 to Ala abolished the protease activity of the light chain, but its ability to bind the polypeptide substrate was retained. Each recombinant light chain could be reconstituted with the heavy chain of **tetanus toxin**, yielding the same level of disulfide-linked species as the two native chains. Whereas the **toxin** formed with wild-type light chain exhibited appreciable neuromuscular paralysis activity and mouse lethality, the equiv. dichain material contg. the Ala234 mutant lacked neurotoxicity in both the in vitro and in vivo assays. Thus, these results demonstrate directly, for the first time, that the lethality of **tetanus toxin** and its inhibition of exocytosis in intact neurons are attributable largely, if not exclusively, to endoprotease activity.

=> s l8 and cellubrevin

L10 2 L8 AND CELLUBREVIN

=> d bib ab 1-2

L10 ANSWER 1 OF 2 CAPLUS COPYRIGHT 1998 ACS
AN 1997:270696 CAPLUS
DN 126:273488
TI **Botulinum** Neurotoxin B Inhibits Insulin-Stimulated Glucose Uptake into 3T3-L1 Adipocytes and Cleaves **Cellubrevin** Unlike Type A **Toxin** Which Failed To Proteolyze the SNAP-23 Present
AU Chen, Fusheng; Foran, Patrick; Shone, Clifford C.; Foster, Keith A.; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1997), 36(19), 5719-5728
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB In this study, exposure of cultured 3T3-L1 adipocytes to **botulinum** neurotoxin (BoNT) B in a low-ionic strength medium was found to block insulin-evoked glucose uptake by up to 64%. BoNT B was shown by immunoblotting to cause extensive proteolysis of **cellubrevin** (Cbr) and synaptobrevin (Sbr) resulting in a significant blockade of the insulin-stimulated translocation of

glucose transporter-isotype 4 (GLUT4) to the plasmalemma. This establishes that these two **toxin** substrates contribute to the insulin-regulated fusion of GLUT4-contg. vesicles with the plasmalemma, at least in this differentiated 3T3-L1 clone. Although SNAP-25 was not detectable in the differentiated adipocytes, its functional homolog SNAP-23 is abundant and largely confined to the plasmalemma. SNAP-23 proved to be resistant to cleavage by BoNT A. Consistent with these results, type A did not block insulin-induced glucose uptake, precluding a demonstration of its likely importance in this process.

L10 ANSWER 2 OF 2 CAPLUS COPYRIGHT 1998 ACS
AN 1995:495141 CAPLUS
DN 122:233062
TI Blockade by **Botulinum** Neurotoxin B of Catecholamine Release from Adrenochromaffin Cells Correlates with Its Cleavage of Synaptobrevin and a Homolog Present on the Granules
AU Foran, Patrick; Lawrence, Gary; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1995), 34(16), 5494-503
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS
AB **Botulinum** neurotoxin type B blocks transmitter release via a selective endoproteolysis of the small clear vesicle membrane protein synaptobrevin that is essential for neuro-exocytosis. In view of the distinct characteristic of exocytosis of adrenochromaffin granules and considering the controversy over the presence of synaptobrevin on the latter, this study aimed to det. the mol. basis of the inhibition by this **toxin** of secretion from chromaffin cells. Thus, affinity-purified antibodies against a synaptobrevin synthetic peptide were used to quantify its concns. in subcellular fractions of bovine adrenal medulla. The latter, as well as d. gradient centrifugation and size-exclusion chromatog., showed that >70% of the protein copurifies with the granules and their marker, dopamine .beta.-hydroxylase. Notably, much lower concns. of synaptobrevin and synaptophysin were found in chromaffin granules than in small clear vesicles (.apprx.9% and .apprx.2%, resp.); however, isolated granule membranes exhibited greater enrichments (.apprx.35% and .apprx.9%). A second immunoreactive protein was colocalized with synaptobrevin on chromaffin granules; in view of its susceptibility to the **toxin** and lower Mr, it is assumed to be **cellubrevin**, and, also, because of its high homol. Involvement of synaptobrevin and **cellubrevin** in Ca2+-triggered granule exocytosis was established by the demonstrated correlation between the extent of **botulinum** neurotoxin B-induced inhibition of secretion and their selective proteolysis following introduction of the **toxin** into intact chromaffin cells. On the basis of these collective findings, it is concluded that these proteins occur on chromaffin granules and one or both are essential for exocytosis.

=> s l8 and neuromuscular

L11 12 L8 AND NEUROMUSCULAR

=> d bib ab 1-12

L11 ANSWER 1 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1996:102543 CAPLUS
DN 124:127109
TI Conjugates of **clostridial** toxins and drugs for use in treatment of **neuromuscular** disorders

IN **Dolly, James Oliver**; Aoki, Kei Roger; Wheeler, Larry Allen; Garst, Michael Elwood
PA Allergan, Inc., USA
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
PI WO 9532738 A1 951207
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-GB1253 950531
PRAI GB 94-10870 940531
GB 94-10871 940531
DT Patent
LA English
AB A chem. conjugate for treating a nerve cell related disorder is provided. This conjugate includes an active or inactive **Clostridial toxin** having specificity for a target nerve cell. The **toxin** is conjugated to a drug or other bioactive mol. without affecting the **toxin**'s ability to enter the target nerve cell. Recombinant Ala-234 **tetanus toxin** L chain mutant was prepd. and a reconstituted **tetanus toxin** dimer prepd. with the L chain mutant and native H chain was shown to be nontoxic. The process of conjugating vesamicol to this reconstituted, inactive **toxin** was described. Mutant **botulinum toxin** A L chains were also prepd. and the reconstituted dimer **toxin** shown to be inactive.

L11 ANSWER 2 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1994:317560 CAPLUS
DN 120:317560
TI A Single Mutation in the Recombinant Light Chain of **Tetanus Toxin** Abolishes Its Proteolytic Activity and Removes the Toxicity Seen after Reconstitution with Native Heavy Chain
AU Li, Yan; Foran, Patrick; Fairweather, Neil F.; de Paiva, Anton; Weller, Ulrich; Dougan, Gordon; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1994), 33(22), 7014-20
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS
AB Specific proteolysis by the **tetanus toxin** light chain of a vesicle-assocd. membrane protein (VAMP) involved in exocytosis is thought to underlie its intracellular blockade of neurotransmitter release. To substantiate this mechanism, recombinant light chain was expressed as a maltose binding protein-light chain fusion product in Escherichia coli. After purifn. by affinity chromatog. and cleavage with factor Xa, the resultant light chain was isolated and its identity confirmed by Western blotting and N-terminal sequencing. It exhibited activity similar to that of the native light chain in proteolyzing its target in isolated bovine small synaptic vesicles and in hydrolyzing a 62-residue synthetic polypeptide spanning the cleavage site of the substrate. The importance of Glu234 in the catalytic activity of the light chain, possibly analogous to Glu143 of thermolysin, was examd. using site-directed mutagenesis. Changing Glu234 to Ala abolished the protease activity of the light chain, but its ability to bind the polypeptide substrate was retained. Each recombinant light chain could be reconstituted with the heavy chain of **tetanus toxin**, yielding the same level of disulfide-linked species as the two native chains. Whereas the

toxin formed with wild-type light chain exhibited appreciable **neuromuscular** paralysis activity and mouse lethality, the equiv. dichain material contg. the Ala234 mutant lacked neurotoxicity in both the in vitro and in vivo assays. Thus, these results demonstrate directly, for the first time, that the lethality of **tetanus toxin** and its inhibition of exocytosis in intact neurons are attributable largely, if not exclusively, to endoprotease activity.

L11 ANSWER 3 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1994:25317 CAPLUS
DN 120:25317
TI **Botulinum** A like type B and **tetanus** toxins fulfils criteria for being a zinc-dependent protease
AU de Paiva, Anton; Ashton, Anthony C.; Foran, Patrick; Schiavo, Giampetro; Montecucco, Cesare; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol. Med., London, UK
SO J. Neurochem. (1993), 61(6), 2338-41
CODEN: JONRA9; ISSN: 0022-3042
DT Journal
LA English
AB Although **botulinum** neurotoxin (BoNT) types A and B and **tetanus toxin** (TeTx) are specific inhibitors of transmitter release whose light chains contain a zinc-binding motif characteristic of metalloendoproteases, only the latter two proteolyze synaptobrevin. Chelation of zinc or its readdn. at high concn. hindered blockade of **neuromuscular** transmission by BoNT/A and B, indicating that type A also acts via a zinc-dependent mechanism. Such treatments prevented proteolysis of synaptobrevin II in rat brain synaptic vesicles by BoNT/B and TeTx but only the activity of the latter was antagonized appreciably by ASQFETS, a peptide spanning their cleavage site. The toxins' neuroparalytic activities were attenuated by phosphoramidon or captopril, inhibitors of certain zinc requiring proteases. However, these agents were ineffective in reducing the toxins' degrdn. of synaptobrevin except that a high concn. of captopril partially blocked the activity of TeTx but not BoNT/B, as also found for these drugs when tested on synaptosomal noradrenaline release. These various criteria establish that a zinc-dependent protease activity underlies the neurotoxicity of BoNT/A, a finding confirmed at motor nerve endings for type B and TeTx. Moreover, the low potencies of captopril and phosphoramidon in counteracting the toxins' effects necessitate the design of improved inhibitors for possible use in the clin. treatment of **tetanus** or botulism.

L11 ANSWER 4 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1994:24998 CAPLUS
DN 120:24998
TI Factors underlying the characteristic inhibition of the neuronal release of transmitters by **tetanus** and various **botulinum** toxins
AU Ashton, Anthony C.; de Paiva, Anton M.; Poulain, Bernard; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol. Med., London, SW7 2AY, UK
SO Botulinum Tetanus Neurotoxins [Proc. Int. Conf.] (1993), Meeting Date 1992, 191-213. Editor(s): Dasgupta, Bibhuti R. Publisher: Plenum, New York, N. Y.
CODEN: 59KIAW
DT Conference; General Review
LA English
AB A review with 77 refs. It has been established that the free thiols play no role in the intoxication by **botulin** A while neither the inter- or intra-chain disulfides contribute to **toxin** binding or its intracellular action. However, the inter-chain is essential for **toxin** internalization. The

physiol. relevant acceptors for **botulin** A, E, F and **tetanus toxin** are distinct at the **neuromuscular** junction and, also, in the CNS. **Botulin** A differs from the other toxins in that a Ca2+ ionophore is able to reverse its action to a much greater extent; also, intact microtubules are not involved in the action of type A, whereas they seem to be required for full intoxication with **botulin** B, E, F and **tetanus toxin**. Notably, the difference in Ca2+ reversal of poisoning in synaptosomes is very similar to findings at the motor nerve endings.

L11 ANSWER 5 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1993:553710 CAPLUS
DN 119:153710
TI A role for the interchain disulfide or its participating thiols in the internalization of **botulinum** neurotoxin A revealed by a **toxin** derivative that binds to ecto-acceptors and inhibits transmitter release intracellularly
AU de Paiva, Anton; Poulain, Bernard; Lawrence, Gary W.; Shone, Clifford C.; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO J. Biol. Chem. (1993), 268(28), 20838-44
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB **Botulinum** neurotoxin type A consists of a disulfide-linked light and heavy chain, with an intradisulfide present within the C-terminal half of the latter. The functional consequences of reducing these bonds and alkylating the thiols were investigated. Modification of free cysteine residues had no effect on the toxicity in mouse bioassays or an acetylcholine release in the mouse nerve-diaphragm and the buccal ganglion of Aplysia californica. However, redn. of the **toxin** prior to alkylation drastically decreased neuroparalytic potency; yet, this deriv. inhibited transmitter release if injected directly into a presynaptic neuron in the Aplysia ganglion or added to bovine permeabilized adrenal chromaffin cells. Its antagonism of the action of **botulinum** neurotoxin A at mammalian motor nerve endings and Aplysia neurons indicates retention of the ability to bind to the **toxin**'s productive ectoacceptors. Thus, the abolition of the toxicity of extracellularly applied **botulinum** neurotoxin A by the cleavage of both disulfides, and the alkylation of the half-cystines involved, results from ineffective uptake. Modified forms of the isolated chains of **botulinum** neurotoxin A were utilized to det. which of the disulfides were necessary for internalization. Alkylation of the cysteines in the light and heavy chains, including those involved in the interchain bond but excluding those of the intact disulfide in the heavy chain, revealed that the intermol. bond must be present, or the thiols concerned unmodified, for **botulinum** neurotoxin A to undergo membrane translocation into Aplysia neurons.

L11 ANSWER 6 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1991:96539 CAPLUS
DN 114:96539
TI Light chain of **botulinum** neurotoxin is active in mammalian motor nerve terminals when delivered via liposomes
AU De Paiva, Anton; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., South Kensington/London, SW7 2AY, UK
SO FEBS Lett. (1990), 277(1-2), 171-4
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English
AB Liposomal encapsulation of the individual light and heavy chain of

botulinum neurotoxin A was used to investigate their intracellular effects on synaptic transmission at the murine **neuromuscular** junction. Bath application to phrenic nerve-hemidiaphragms of liposomes contg. heavy chain (up to 75 nM) caused no alteration in neurally-evoked muscle tension. In contrast, liposomes with entrapped light chain (9-20 nM final concn.) gave a presynaptic blockade of **neuromuscular** transmission that could be relieved temporarily by 4-aminopyridine, as for the dichain **toxin**. Any contribution from contaminating intact **toxin** was excluded both by the purity and minimal toxicity in mice of the light chain prepns. used, and by the lack of **neuromuscular** paralysis seen with liposomes contg. the max. amt. of native **toxin** that apparently was present in the light chain liposomes. As bath application of high concns. of light chain in the absence of liposomes failed to affect neurotransmitter release, it is concluded that this chain alone can mimic the action of the whole **toxin** inside mammalian motor nerve endings, its predominant site of action. Thus, light chain provides a more effective probe for an intracellular component concerned with Ca2+-dependent secretion.

L11 ANSWER 7 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1989:626960 CAPLUS
DN 111:226960
TI Multiple domains of **botulinum** neurotoxin contribute to its inhibition of transmitter release in Aplysia neurons
AU Poulain, Bernard; Wadsworth, Jonathan D. F.; Shone, Clifford C.; Mochida, Sumiko; Lande, Simon; Melling, Jack; **Dolly, J. Oliver**; Tauc, Ladislav
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, F-91198, Fr.
SO J. Biol. Chem. (1989), 264(36), 21928-33
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The binding, internalization, and inhibition of transmitter release by **botulinum** neurotoxin (BoNT) was investigated using the intact **toxin**, its heavy (HC) or light (LC) chains, and a proteolytic fragment thereof. In Aplysia neurons, blockade of acetylcholine release upon external application of BoNT types A or E was prevented by reducing the temp. to 10.degree., due to arresting intoxication at the membrane binding step. At this low temp., type A HC, H2 (comprised of the N-terminal of HC), or H2L (H2 disulfide-linked to LC) antagonized the neuroparalytic action of BoNT A or E, indicating that the latter bind saturably to common ecto-acceptor via the H2 region. In contrast, H2L was unable to counteract BoNT-induced paralysis at the murine **neuromuscular** junction. In accordance with this species difference, unlike native BoNT, saturable binding of 125I-labeled H2L could not be detected in mammalian peripheral or central nerve terminals. Possibly, there are more stringent structural requirements that form the basis of the **toxin**'s greater effectiveness in inhibiting neurotransmission at the mouse nerve muscle synapses than the Aplysia nerve terminus. In further identification of functional domain in the **toxin**, an unprocessed single-chain form of BoNT type E was ineffective when applied extra- or intracellularly to Aplysia neurons. Notably, bath application of the latter to a neuron preinjected with HC, but not H2L or LC, resulted in a blockade of release. This shows that the single-chain species can become internalized and requires, not only LC, but also processed HC for its inhibitory action; consequently, the proteolyzed form of BoNT E was active.

L11 ANSWER 8 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1989:610381 CAPLUS

DN 111:210381
TI Inhibition of transmitter release by **botulinum** neurotoxin A. Contribution of various fragments to the intoxication process
AU Poulain, Bernard; Wadsworth, Jonathan D. F.; Maisey, E. Anne; Shone, Clifford C.; Melling, Jack; Tauc, Ladislav; **Dolly, J. Oliver**
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, Fr.
SO Eur. J. Biochem. (1989), 185(1), 197-203
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB The contribution of a proteolytic fragment (H2L) of **botulinum** neurotoxin type A (comprised of the amino-terminal region of the heavy-chain disulfide-linked to the light chain) to inhibition of neurotransmitter release was investigated, using central cholinergic synapses of Aplysia, rodent nerve-diaphragm prepns. and cerebrocortical synaptosomes. No redn. in neurotransmitter release was obsd. following external application to these prepns. of highly purified H2L or after intracellular injection into Aplysia neurons. The lack of activity was not the result of alteration in the light chain of H2L during prepn. of the latter because renaturation of this light chain was intact heavy chain produced a toxic di-chain form and simultaneous application of heavy and light chains from H2L inhibited transmitter release in Aplysia. Bath application of H2L and heavy chain together inhibited release of transmitter; however, at the **neuromuscular** junction the potency of this mixt. was much lower than that of native **toxin**. A similar blockade resulted when heavy chain was applied intracellularly and H2L added to the bath, demonstrating that H2L is taken up into cholinergic neurons of Aplysia. This uptake is shown to be mediated by the amino-terminal moiety of heavy chain (H2), because bath application of light chain plus H2 led to a decrease in acetylcholine release from a neuron that had been injected with heavy chain. A role within the neuron is implicated for a carboxy terminal portion of heavy chain (H1) since intracellular injection of light chain and H2 did not affect transmitter release. Although the situation is unclear in mammalian nerves, these collective findings indicate that blockade of transmitter release in Aplysia neurons requires the intracellular presence of light chain and H1 (by inference), whilst H2 contributes to the internalization step.

L11 ANSWER 9 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1989:19639 CAPLUS
DN 110:19639
TI Involvement of the constituent chains of **botulinum** neurotoxins A and B in the blockade of neurotransmitter release
AU Maisey, E. Anne; Wadsworth, Jonathan D. F.; Poulain, Bernard; Shone, Clifford C.; Melling, Jack; Gibbs, Paul; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AY, UK
SO Eur. J. Biochem. (1988), 177(3), 683-91
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB The abilities of **botulinum** neurotoxins, types A and B (single and two-chain forms) to inactivate an intraneuronal component required for transmitter release were quantified in a phrenic-nerve-diaphragm prepn., cerebrocortical synaptosomes or the buccal ganglion of Aplysia californica, and compared with the mouse toxicity assay. Homogeneous prepns. of the individually renatured polypeptide chains of both **toxin** types showed low residual toxicity in the whole animal and had no effect on neurotransmission in all three systems, when tested singly. Mixts. of individually renatured heavy chain, from type A or B, and either light chain

proved very effective in blocking the evoked release of acetylcholine when bath-applied to the buccal ganglion of Aplysia whereas they were relatively inactive on mammalian nerve terminals, indicating a less efficient uptake of the polypeptides in the latter. When renatured together, the homologous, but not the heterologous, chains of each **toxin** yielded toxic, disulfide-linked two-chain species. A role for the heavy chain alone in acceptor recognition and membrane translocation was implicated by the blockade of acetycholine release produced when light chain was applied to a ganglion of Aplysia previously bathed in heavy chain and washed extensively. No blockade was obsd. when the order of application of the two chains was reversed. These findings are discussed in the context of the intracellular requirement for both the constituent **toxin** chains for toxicity, and in the apparent need for these chains to be linked via a disulfide bond for uptake in rodents but not in Aplysia.

L11 ANSWER 10 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1988:468593 CAPLUS
DN 109:68593
TI Roles of the constituent chains of **botulinum** neurotoxin type A in the blockade of **neuromuscular** transmission in mice
AU Wadsworth, Jonathan D. F.; Shone, Clifford C.; Melling, Jack; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol., London, SW7 2AZ, UK
SO Biochem. Soc. Trans. (1988), 16(5), 886-7
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English
AB The effects of **botulin** A light chain (LC) and heavy chain (HC) on nerve-stimulated muscle contraction was examd. in mouse phrenic nerve hemodiaphragms; bath application of LC or HC to supramaximally stimulated hemidiaphragm prepns. failed to produce a change in nerve-evoked twitch tension. Bath application of HC followed by LC 10 min later elicited a blockage of transmission equiv. to that produced by low concns. of **botulin** A. When the prepns. were incubated with HC followed by **botulin** A, no significant change was found compared to application of **botulin** A alone. Thus both HC and LC are required in the bath to produce neuroparalysis.

L11 ANSWER 11 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1986:529146 CAPLUS
DN 105:129146
TI Interaction of iodine-125-labeled **botulinum** neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves
AU Black, Jennifer D.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO J. Cell Biol. (1986), 103(2), 521-34
CODEN: JCLBA3; ISSN: 0021-9525
DT Journal
LA English
AB The labeling patterns produced by radioiodinated **botulinum** neurotoxin (125I-BoNT) types A and B at the vertebrate **neuromuscular** junction were investigated using electron microscopic autoradiog. 125I-BoNT type A, applied in vivo or in vitro to mouse diaphragm or frog cutaneous pectoris muscle, interacts saturably with the motor nerve terminal only; Ag grains occur on the plasma membrane, within the synaptic bouton, and in the axoplasm of the nerve trunk, suggesting internalization and retrograde intraaxonal transport of **toxin** or fragments thereof. This result is reconcilable with the similar, but not

identical, pharmacol. action of these **toxin** types. The saturability of labeling in each case suggested the involvement of acceptors; on preventing the internalization step with metabolic inhibitors, their precise location became apparent. They were found on all unmyelinated areas of the nerve terminal membrane, including the preterminal axon and the synaptic bouton. Although 125I-BoNT type A interacts specifically with developing terminals of newborn rats, the unmyelinated plasma membrane of the nerve trunk is not labeled, indicating that the acceptors are unique components restricted to the nerve terminal area. BoNT types A and B have distinct acceptors on the terminal membrane. Having optimized the conditions for satn. of these binding sites and calibrated the autoradiog. procedure, the densities of the acceptors for types A and B were .apprx.150 and 630/.mu.m2 of membrane, resp. Presumably these membrane acceptors target BoNT to the nerve terminal and mediate its delivery to an intracellular site, thus contributing to the toxins selective inhibitory action on neurotransmitter release.

L11 ANSWER 12 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1983:29377 CAPLUS
DN 98:29377
TI Preparation of neurotoxic 3H-.beta.-bungarotoxin: demonstration of saturable binding to brain synapses and its inhibition by **toxin** I
AU Othman, Iekhsan B.; Spokes, John W.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, UK
SO Eur. J. Biochem. (1982), 128(1), 267-76
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
AB Homogeneous .beta.-bungarotoxin [12778-32-4] was radiolabeled with N-succinimidyl-[2,3-3H]propionate. Stable, dipropionylated material was obtained which was tritiated on both subunits and had a specific radioactivity of 102 Ci/mmol. After sepn. from unlabeled **toxin** by isoelec. focussing, it exhibited significant biol. activity in both the peripheral and central nervous systems but had negligible phospholipase A2 [9001-84-7] activity towards lecithin or cerebrocortical synaptosomes. The labeled neurotoxin binds specifically to a single class of noninteracting sites of high affinity (Kd = 0.6 nM) on rat cerebral cortex synaptosomes; the content of sites is .apprx.150 fmol/mg protein. This binding was inhibited by unlabeled .beta.-bungarotoxin with a potency which indicates that tritiation does not alter the affinity significantly. The assocn. of **toxin** with its binding component and its dissocn. were monophasic; rate consts. obsd. were 7.8 .times. 105M-1/s and 5.6 .times. 10-4/s at 37.degree., resp. .beta.-Bungarotoxin whose phospholipase activity had been inactivated with p-bromophenacyl bromide inhibited to some extent the binding of tritiated **toxin** but with low efficacy. taipoxin [52019-39-3] And phospholipase A2 from bee venom, but not Naja melanoleuca, inhibited the synaptosomal binding of **toxin** with low potencies in the presence, but not the absence, of Ca2+. **Toxin** I, a single-chain protein from Dendroaspis polylepis known to potentiate transmitter release at chick **neuromuscular** junction, completely inhibited the binding of [3H].beta.-bungarotoxin with a Ki of 0.07 nM; this explains its ability to antagonize the neuroparalytic action of .beta.-bungarotoxin. Other pure presynaptic neurotoxins, .alpha.-latrotoxin [65988-34-3] and **botulinum** neurotoxin failed to antagonize the obsd. binding; likewise tityustoxin [39465-37-7], which is known to affect Na channels, had no effect on [3H].beta.-bungarotoxin binding. Trypsinization of synaptosomes completely destroyed the binding activity, suggesting that the binding component is a protein; the functional role of the latter is discussed in relation to the specificity of **toxin** binding.

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     (FILE 'HOME' ENTERED AT 08:57:05 ON 09 MAR 1998)

     FILE 'EMBASE, MEDLINE, BIOSIS, BIOTECHDS, LIFESCI, CONFSCI, WPIDS,
     JAPIO, DISSABS, CAPLUS' ENTERED AT 08:57:56 ON 09 MAR 1998
L1          271 S CELLUBREVIN
L2          108 S L1 AND (TETANUS OR BOTULINUM)
L3           18 S L2 AND NEUROTRANSMIT?
L4            8 DUP REM L3 (10 DUPLICATES REMOVED)
                E DOLLY JAMES OLIVER/AU
L5          109 S E1 OR E3
L6           62 S L5 AND TOXIN
L7           62 DUP REM L6 (0 DUPLICATES REMOVED)
L8           40 S L7 AND (CLOSTRID? OR BOTULIN? OR TETANUS)
L9            4 S L8 AND VAMP
L10           2 S L8 AND CELLUBREVIN
L11          12 S L8 AND NEUROMUSCULAR

=> d l7 bib 1-62


L7   ANSWER 1 OF 62  CAPLUS  COPYRIGHT 1998 ACS
AN   1997:361532  CAPLUS
DN   127:1851
TI   Site-Directed Mutagenesis of Dendrotoxin Reveals Amino Acids
     Critical for its Interaction with Neuronal K+ Channels
AU   Smith, Leonard A.; Reid, Paul F.; Wang, Fan C.; Parcej, David N.;
     Schmidt, James J.; Olson, Mark A.; Dolly, J. Oliver
CS   Department of Immunology and Molecular Biology Toxinology Division,
     United States Army Medical Research Institute of Infectious
     Diseases, Frederick, MD, 21702-5011, USA
SO   Biochemistry (1997), 36(25), 7690-7696
     CODEN: BICHAW; ISSN: 0006-2960
PB   American Chemical Society
DT   Journal
LA   English
OS   CJACS-IMAGE; CJACS

L7   ANSWER 2 OF 62  CAPLUS  COPYRIGHT 1998 ACS
AN   1997:270696  CAPLUS
DN   126:273488
TI   Botulinum Neurotoxin B Inhibits Insulin-Stimulated Glucose Uptake
     into 3T3-L1 Adipocytes and Cleaves Cellubrevin Unlike Type A
     Toxin Which Failed To Proteolyze the SNAP-23 Present
AU   Chen, Fusheng; Foran, Patrick; Shone, Clifford C.; Foster, Keith A.;
     Dolly, J. Oliver
CS   Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO   Biochemistry (1997), 36(19), 5719-5728
     CODEN: BICHAW; ISSN: 0006-2960
PB   American Chemical Society
DT   Journal
LA   English
OS   CJACS-IMAGE; CJACS

L7   ANSWER 3 OF 62  CAPLUS  COPYRIGHT 1998 ACS
AN   1997:135095  CAPLUS
DN   126:169620
TI   Importance of two adjacent C-terminal sequences of SNAP-25 in
     exocytosis from intact and permeabilized chromaffin cells revealed
     by inhibition with botulinum neurotoxins A and E
AU   Lawrence, Gary W.; Foran, Patrick; Mohammed, N.; DasGupta, B. R.;
     Dolly, J. Oliver
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CS Department of Biochemistry, Imperial College of Science Technology and Medicine, London, SW7 2AY, UK
SO Biochemistry (1997), 36(11), 3061-3067
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
OS CJACS-IMAGE; CJACS

L7 ANSWER 4 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1997:280364 CAPLUS
DN 126:260299
TI Microtubules and microfilaments participate in the inhibition of synaptosomal noradrenaline release by tetanus **toxin**. [Erratum to document cited in CA126:114455]
AU Ashton, Anthony C.; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, UK
SO J. Neurochem. (1997), 68(5), 2225
CODEN: JONRA9; ISSN: 0022-3042
PB Lippincott-Raven
DT Journal
LA English

L7 ANSWER 5 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1997:82866 CAPLUS
DN 126:114455
TI Microtubules and microfilaments participate in the inhibition of synaptosomal noradrenaline release by tetanus **toxin**
AU Ashton, Anthony C.; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, UK
SO J. Neurochem. (1997), 68(2), 649-658
CODEN: JONRA9; ISSN: 0022-3042
PB Lippincott-Raven
DT Journal
LA English

L7 ANSWER 6 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1997:604084 CAPLUS
DN 127:230498
TI Seizures and hippocampal damage produced by dendrotoxin-K in rats is prevented by the 21-aminosteroid U-74389G
AU Bagetta, Giacinto; Palma, Ernesto; Piccirilli, Silvia; Nistico, Giuseppe; **Dolly, James Oliver**
CS Department Pharmacobiology, University Calabria, Cosenza, Italy
SO Exp. Neurol. (1997), 147(1), 204-210
CODEN: EXNEAC; ISSN: 0014-4886
PB Academic
DT Journal
LA English

L7 ANSWER 7 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1996:87898 CAPLUS
DN 124:109403
TI BoNT/C1 Cleaves both Syntaxin and SNAP-25 in Intact and Permeabilized Chromaffin Cells: Correlation with Its Blockade of Catecholamine Release
AU Foran, Patrick; Lawrence, Gary W.; Shone, Clifford C.; Foster, Keith; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1996), 35(8), 2630-6
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS

L7 ANSWER 8 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1996:197576 CAPLUS
DN 124:285094
TI Distinct exocytotic responses of intact and permeabilised chromaffin cells after cleavage of the 25-kDa synaptosomal-associated protein (SNAP-25) or synaptobrevin by botulinum **toxin** A or B
AU Lawrence, Gary W.; Foran, Patrick; **Dolly, J. Oliver**
CS Dep. Biochem., Imperial College Science, Technology and Medicine, London, UK
SO Eur. J. Biochem. (1996), 236(3), 877-86
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 9 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1996:102543 CAPLUS
DN 124:127109
TI Conjugates of clostridial toxins and drugs for use in treatment of neuromuscular disorders
IN **Dolly, James Oliver**; Aoki, Kei Roger; Wheeler, Larry Allen; Garst, Michael Elwood
PA Allergan, Inc., USA
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
PI WO 9532738 A1 951207
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-GB1253 950531
PRAI GB 94-10870 940531
GB 94-10871 940531
DT Patent
LA English

L7 ANSWER 10 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1996:29137 CAPLUS
DN 124:79171
TI Tetanus **toxin** inhibits neuroexocytosis even when its Zn2+-dependent protease activity is removed
AU Ashton, Anthony C.; Li, Yan; Doussau, Frederic; Weller, Ullrich; Dougan, Gordon; Poulain, Bernard; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. College, London, SW7 2AY, UK
SO J. Biol. Chem. (1995), 270(52), 31386-90
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L7 ANSWER 11 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1995:495141 CAPLUS
DN 122:233062
TI Blockade by Botulinum Neurotoxin B of Catecholamine Release from Adrenochromaffin Cells Correlates with Its Cleavage of Synaptobrevin and a Homolog Present on the Granules
AU Foran, Patrick; Lawrence, Gary; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1995), 34(16), 5494-503
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS

L7 ANSWER 12 OF 62 CAPLUS COPYRIGHT 1998 ACS

AN 1995:220501 CAPLUS
DN 122:3157
TI Differences in the Protease Activities of Tetanus and Botulinum B Toxins Revealed by the Cleavage of Vesicle-Associated Membrane Protein and Various Sized Fragments
AU Foran, Patrick; Shone, Clifford C.; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1994), 33(51), 15365-74
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS

L7 ANSWER 13 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:317560 CAPLUS
DN 120:317560
TI A Single Mutation in the Recombinant Light Chain of Tetanus **Toxin** Abolishes Its Proteolytic Activity and Removes the Toxicity Seen after Reconstitution with Native Heavy Chain
AU Li, Yan; Foran, Patrick; Fairweather, Neil F.; de Paiva, Anton; Weller, Ulrich; Dougan, Gordon; **Dolly, J. Oliver**
CS Department of Biochemistry, Imperial College, London, SW7 2AY, UK
SO Biochemistry (1994), 33(22), 7014-20
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS

L7 ANSWER 14 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:452037 CAPLUS
DN 121:52037
TI Botulinum A and the light chain of tetanus toxins inhibit distinct stages of Mg . ATP-dependent catecholamine exocytosis from permeabilized chromaffin cells
AU Lawrence, Gary W.; Weller, Ulrich; **Dolly, J. Oliver**
CS Biochem. Dep., Imperial Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO Eur. J. Biochem. (1994), 222(2), 325-33
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 15 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:98963 CAPLUS
DN 120:98963
TI Antagonism of the intracellular action of botulinum neurotoxin type A with monoclonal antibodies that map to light-chain epitopes
AU Cenci di Bello, Isabelle; Poulain, Bernard; Shone, Clifford C.; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem, Imp. Coll. Sci., Technol Med., London, UK
SO Eur. J. Biochem. (1994), 219(1-2), 161-9
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 16 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:570660 CAPLUS
DN 121:170660
TI Probing the process of transmitter release with botulinum and tetanus neurotoxins
AU **Dolly, J. Oliver**; Paiva, Anton de; Foran, Patrick; Lawrence, Gary; Daniels-Holgate, Phillipa; Ashton, Anthony C.
CS Department Biochemistry, Imperial College, London, SW7 2AZ, UK
SO Semin. Neurosci. (1994), 6(3), 149-58
CODEN: SNEUEZ; ISSN: 1044-5765

DT Journal; General Review
LA English

L7 ANSWER 17 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1993:553710 CAPLUS
DN 119:153710
TI A role for the interchain disulfide or its participating thiols in the internalization of botulinum neurotoxin A revealed by a **toxin** derivative that binds to ecto-acceptors and inhibits transmitter release intracellularly
AU de Paiva, Anton; Poulain, Bernard; Lawrence, Gary W.; Shone, Clifford C.; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO J. Biol. Chem. (1993), 268(28), 20838-44
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L7 ANSWER 18 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1993:249609 CAPLUS
DN 118:249609
TI Cloning and functional expression of dendrotoxin K from black mamba, a potassium channel blocker
AU Smith, Leonard A.; Lafaye, Pierre J.; LaPenotiere, Hugh F.; Spain, Tara; **Dolly, J. Oliver**
CS Dep. Immunol. Mol. Biol., U. S. Army Med. Res. Inst. Infect. Dis., Frederick, MD, 21702-5011, USA
SO Biochemistry (1993), 32(21), 5692-7
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
OS CJACS-IMAGE; CJACS

L7 ANSWER 19 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:25317 CAPLUS
DN 120:25317
TI Botulinum A like type B and tetanus toxins fulfils criteria for being a zinc-dependent protease
AU de Paiva, Anton; Ashton, Anthony C.; Foran, Patrick; Schiavo, Giampetro; Montecucco, Cesare; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol. Med., London, UK
SO J. Neurochem. (1993), 61(6), 2338-41
CODEN: JONRA9; ISSN: 0022-3042
DT Journal
LA English

L7 ANSWER 20 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1994:24998 CAPLUS
DN 120:24998
TI Factors underlying the characteristic inhibition of the neuronal release of transmitters by tetanus and various botulinum toxins
AU Ashton, Anthony C.; de Paiva, Anton M.; Poulain, Bernard; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol. Med., London, SW7 2AY, UK
SO Botulinum Tetanus Neurotoxins [Proc. Int. Conf.] (1993), Meeting Date 1992, 191-213. Editor(s): Dasgupta, Bibhuti R. Publisher: Plenum, New York, N. Y.
CODEN: 59KIAW
DT Conference; General Review
LA English

L7 ANSWER 21 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1992:565669 CAPLUS
DN 117:165669
TI Tetanus **toxin** and botulinum toxins type A and B inhibit

glutamate, .gamma.-aminobutyric acid, aspartate, and Met-enkephalin release from synaptosomes. Clues to the locus of action
AU McMahon, Harvey T.; Foran, Patrick; **Dolly, J. Oliver**; Verhage, Matthijs; Wiegant, Victor M.; Nicholls, David G.
CS Dep. Biochem., Univ. Dundee, Dundee, DD1 4HN, UK
SO J. Biol. Chem. (1992), 267(30), 21338-43
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L7 ANSWER 22 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1992:442411 CAPLUS
DN 117:42411
TI Differences in the temperature dependencies of uptake of botulinum and tetanus toxins in Aplysia neurons
AU Poulain, Bernard; De Paiva, Anton; **Dolly, J. Oliver**; Weller, Ulrich; Tauc, Ladislav
CS Lab. Neurobiol. Cell. Mol., CNRS, Gif-sur-Yvette, 91198, Fr.
SO Neurosci. Lett. (1992), 139(2), 289-92
CODEN: NELED5; ISSN: 0304-3940
DT Journal
LA English

L7 ANSWER 23 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1992:484961 CAPLUS
DN 117:84961
TI Production of seizures and brain damage in rats by .alpha.-dendrotoxin, a selective potassium channel blocker
AU Bagetta, Giacinto; Nistico, Giuseppe; **Dolly, J. Oliver**
CS Dep. Biol., Univ. Rome, Rome, Italy
SO Neurosci. Lett. (1992), 139(1), 34-40
CODEN: NELED5; ISSN: 0304-3940
DT Journal
LA English

L7 ANSWER 24 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1993:249950 CAPLUS
DN 118:249950
TI Cloning of a bovine voltage-gated potassium channel gene utilizing partial amino acid sequence of a dendrotoxin-binding protein from brain cortex
AU Reid, Paul F.; Pongs, Olaf; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AY, UK
SO FEBS Lett. (1992), 302(1), 31-4
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English

L7 ANSWER 25 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1991:443913 CAPLUS
DN 115:43913
TI Heterologous combinations of heavy and light chains from botulinum neurotoxin A and tetanus **toxin** inhibit neurotransmitter release in Aplysia
AU Poulain, Bernard; Mochida, Sumiko; Weller, Ulrich; Hogy, Barbara; Habermann, Ernst; Wadsworth, Jonathan D. F.; Shone, Clifford C.; **Dolly, J. Oliver**; Tauc, Ladislav
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, F-91198, Fr.
SO J. Biol. Chem. (1991), 266(15), 9580-5
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L7 ANSWER 26 OF 62 CAPLUS COPYRIGHT 1998 ACS

AN 1991:137770 CAPLUS
DN 114:137770
TI Microtubule-dissociating drugs and A 23187 reveal differences in the inhibition of synaptosomal transmitter release by botulinum neurotoxins types A and B
AU Ashton, Anthony C.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO J. Neurochem. (1991), 56(3), 827-35
CODEN: JONRA9; ISSN: 0022-3042
DT Journal
LA English

L7 ANSWER 27 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1990:547037 CAPLUS
DN 113:147037
TI ADP-ribosylation of cerebrocortical synaptosomal proteins by cholera, pertussis and botulinum toxins
AU Ashton, Anthony C.; Edwards, Kathryn; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol. Med., London, UK
SO Toxicon (1990), 28(8), 963-73
CODEN: TOXIA6; ISSN: 0041-0101
DT Journal
LA English

L7 ANSWER 28 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1990:71979 CAPLUS
DN 112:71979
TI Characterization of binding sites for .delta.-dendrotoxin in guinea pig synaptosomes: relationship to acceptors for the potassium-channel probe .alpha.-dendrotoxin
AU Muniz, Zilda M.; Diniz, Carlos R.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO J. Neurochem. (1990), 54(1), 343-6
CODEN: JONRA9; ISSN: 0022-3042
DT Journal
LA English

L7 ANSWER 29 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1990:193514 CAPLUS
DN 112:193514
TI Calcium-dependent noradrenaline release from permeabilized PC12 cells is blocked by botulinum neurotoxin A or its light chain
AU McInnes, Colin; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., London, SW7 2AY, UK
SO FEBS Lett. (1990), 261(2), 323-6
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English

L7 ANSWER 30 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1991:625820 CAPLUS
DN 115:225820
TI Inhibition of neurotransmitter release by botulinum neurotoxins and tetanus **toxin** at Aplysia synapses: role of the constituent chains
AU Poulain, Bernard; Mochida, Sumiko; Wadsworth, Jonathan D. F.; Weller, Ulrich; Habermann, Ernst; **Dolly, J. Oliver**; Tauc, Ladislav
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, 91198, Fr.
SO J. Physiol. (Paris) (1990), 84(4), 247-61
CODEN: JOPHAN; ISSN: 0021-7948
DT Journal
LA English

L7 ANSWER 31 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1991:96539 CAPLUS
DN 114:96539
TI Light chain of botulinum neurotoxin is active in mammalian motor nerve terminals when delivered via liposomes
AU De Paiva, Anton; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci., Technol. Med., South Kensington/London, SW7 2AY, UK
SO FEBS Lett. (1990), 277(1-2), 171-4
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English

L7 ANSWER 32 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1989:626960 CAPLUS
DN 111:226960
TI Multiple domains of botulinum neurotoxin contribute to its inhibition of transmitter release in Aplysia neurons
AU Poulain, Bernard; Wadsworth, Jonathan D. F.; Shone, Clifford C.; Mochida, Sumiko; Lande, Simon; Melling, Jack; **Dolly, J. Oliver**; Tauc, Ladislav
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, F-91198, Fr.
SO J. Biol. Chem. (1989), 264(36), 21928-33
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L7 ANSWER 33 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1989:53330 CAPLUS
DN 110:53330
TI Interactions between discrete neuronal membrane binding sites for the putative potassium channel ligands .beta.-bungarotoxin, dendrotoxin and mast cell degranulating peptide
AU Breeze, Alexander L.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AY, UK
SO Eur. J. Biochem. (1989), 178(3), 771-8
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 34 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1989:610381 CAPLUS
DN 111:210381
TI Inhibition of transmitter release by botulinum neurotoxin A. Contribution of various fragments to the intoxication process
AU Poulain, Bernard; Wadsworth, Jonathan D. F.; Maisey, E. Anne; Shone, Clifford C.; Melling, Jack; Tauc, Ladislav; **Dolly, J. Oliver**
CS Lab. Neurobiol. Cell. Mol., Cent. Natl. Rech. Sci., Gif-sur-Yvette, Fr.
SO Eur. J. Biochem. (1989), 185(1), 197-203
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 35 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1988:468593 CAPLUS
DN 109:68593
TI Roles of the constituent chains of botulinum neurotoxin type A in the blockade of neuromuscular transmission in mice
AU Wadsworth, Jonathan D. F.; Shone, Clifford C.; Melling, Jack; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol., London, SW7 2AZ, UK
SO Biochem. Soc. Trans. (1988), 16(5), 886-7
CODEN: BCSTB5; ISSN: 0300-5127

DT Journal
LA English

L7 ANSWER 36 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1989:19639 CAPLUS
DN 110:19639
TI Involvement of the constituent chains of botulinum neurotoxins A and B in the blockade of neurotransmitter release
AU Maisey, E. Anne; Wadsworth, Jonathan D. F.; Poulain, Bernard; Shone, Clifford C.; Melling, Jack; Gibbs, Paul; Tauc, Ladislav; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AY, UK
SO Eur. J. Biochem. (1988), 177(3), 683-91
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 37 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1988:126336 CAPLUS
DN 108:126336
TI Distribution of acceptors for .beta.-bungarotoxin in the central nervous system of the rat
AU Pelchen-Matthews, Annegret; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol., London, SW7 2AZ, UK
SO Brain Res. (1988), 441(1-2), 127-38
CODEN: BRREAP; ISSN: 0006-8993
DT Journal
LA English

L7 ANSWER 38 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1988:624390 CAPLUS
DN 109:224390
TI Relationship of acceptors for botulinum neurotoxins (types A and B) in rat CNS with the cholinergic marker, Chol-I
AU Evans, David M.; Richardson, Peter J.; Fine, Alan; Mason, William T.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AY, UK
SO Neurochem. Int. (1988), 13(1), 25-36
CODEN: NEUIDS; ISSN: 0197-0186
DT Journal
LA English

L7 ANSWER 39 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1987:453880 CAPLUS
DN 107:53880
TI Botulinum **toxin** A blocks glutamate exocytosis from guinea pig cerebral cortical synaptosomes
AU Sanzhez-Prieto, Jose; Sihra, Talvinder S.; Evans, David; Ashton, Anthony; **Dolly, J. Oliver**; Nicholls, David G.
CS Dep. Biochem., Univ. Dundee, Dundee, DD1 4HN, UK
SO Eur. J. Biochem. (1987), 165(3), 675-81
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 40 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:456031 CAPLUS
DN 105:56031
TI Two acceptor sub-types for dendrotoxin in chick synaptic membranes distinguishable by .beta.-bungarotoxin
AU Black, Adrian R.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Eur. J. Biochem. (1986), 156(3), 609-17
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal

LA English

L7 ANSWER 41 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:529147 CAPLUS
DN 105:129147
TI Interaction of iodine-125-labeled botulinum neurotoxins with nerve terminals. II. Autoradiographic evidence for its uptake into motor nerves by acceptor-mediated endocytosis
AU Black, Jennifer D.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO J. Cell Biol. (1986), 103(2), 535-44
CODEN: JCLBA3; ISSN: 0021-9525
DT Journal
LA English

L7 ANSWER 42 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:529146 CAPLUS
DN 105:129146
TI Interaction of iodine-125-labeled botulinum neurotoxins with nerve terminals. I. Ultrastructural autoradiographic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves
AU Black, Jennifer D.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO J. Cell Biol. (1986), 103(2), 521-34
CODEN: JCLBA3; ISSN: 0021-9525
DT Journal
LA English

L7 ANSWER 43 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:63863 CAPLUS
DN 104:63863
TI The mechanism of action of .beta.-bungarotoxin at the presynaptic plasma membrane
AU Rugolo, Michela; **Dolly, J. Oliver**; Nicholls, David G.
CS Ninewells Med. Sch., Univ. Dundee, Dundee, DD1 9SY, UK
SO Biochem. J. (1986), 233(2), 519-23
CODEN: BIJOAK; ISSN: 0306-3275
DT Journal
LA English

L7 ANSWER 44 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:124640 CAPLUS
DN 104:124640
TI Central action of dendrotoxin: selective reduction of a transient K conductance in hippocampus and binding to localized acceptors
AU Halliwell, James V.; Othman, Iekhsan B.; Pelchen-Matthews, Annegret; **Dolly, J. Oliver**
CS Neuropharmacol. Res. Group, Sch. Pharm., London, WC1 1AX, UK
SO Proc. Natl. Acad. Sci. U. S. A. (1986), 83(2), 493-7
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English

L7 ANSWER 45 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:104029 CAPLUS
DN 104:104029
TI Botulinum neurotoxin type B. Its purification, radioiodination and interaction with rat brain synaptosomal membranes
AU Evans, David M.; Williams, Richard S.; Shone, Clifford C.; Hambleton, Peter; Melling, Jack; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Eur. J. Biochem. (1986), 154(2), 409-16
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal

LA English

L7 ANSWER 46 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1986:456051 CAPLUS
DN 105:56051
TI Involvement of neuronal acceptors for dendrotoxin in its convulsive action in rat brain
AU Black, Adrian R.; Breeze, Alexander L.; Othman, Iekhsan B.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Biochem. J. (1986), 237(2), 397-404
CODEN: BIJOAK; ISSN: 0306-3275
DT Journal
LA English

L7 ANSWER 47 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1985:418126 CAPLUS
DN 103:18126
TI A functional membranous acceptor for dendrotoxin in rat brain: solubilization of the binding component
AU Mehraban, Fuad; Black, Adrian R.; Breeze, Alexander L.; Green, David G.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol., London, SW7 2AZ, UK
SO Biochem. Soc. Trans. (1985), 13(2), 507-8
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English

L7 ANSWER 48 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1985:482826 CAPLUS
DN 103:82826
TI A sensitive and useful radioimmunoassay for neurotoxin and its hemagglutinin complex from Clostridium botulinum
AU Ashton, Anthony C.; Crowther, John S.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Toxicon (1985), 23(2), 235-46
CODEN: TOXIA6; ISSN: 0041-0101
DT Journal
LA English

L7 ANSWER 49 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1984:81014 CAPLUS
DN 100:81014
TI Acceptors for botulinum neurotoxin reside on motor nerve terminals and mediate its internalization
AU **Dolly, J. Oliver**; Black, Jennifer; Williams, Richard S.; Melling, Jack
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Nature (London) (1984), 307(5950), 457-60
CODEN: NATUAS; ISSN: 0028-0836
DT Journal
LA English

L7 ANSWER 50 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1984:565184 CAPLUS
DN 101:165184
TI Identification by crosslinking of a neuronal acceptor protein for dendrotoxin, a convulsant polypeptide
AU Mehraban, Fuad; Breeze, Alexander L.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll. Sci. Technol., London, SW7 2AZ, UK
SO FEBS Lett. (1984), 174(1), 116-22
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English

L7 ANSWER 51 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1984:83928 CAPLUS
DN 100:83928
TI Properties of monoclonal antibodies to nicotinic acetylcholine receptor from chick muscle
AU Mehraban, Fuad; Kemshead, John T.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Eur. J. Biochem. (1984), 138(1), 53-61
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 52 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1983:552783 CAPLUS
DN 99:152783
TI Synaptic binding sites in brain for [3H].beta.-bungarotoxin - a specific probe that perturbs transmitter release
AU Othman, Iekhsan B.; Wilkin, Graham P.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Neurochem. Int. (1983), 5(4), 487-96
CODEN: NEUIDS; ISSN: 0197-0186
DT Journal
LA English

L7 ANSWER 53 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1983:156161 CAPLUS
DN 98:156161
TI Radioiodination of botulinum neurotoxin type A with retention of biological activity and its binding to brain synaptosomes
AU Williams, Richard S.; Tse, Chun Kee; **Dolly, J. Oliver**; Hambleton, Peter; Melling, Jack
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Eur. J. Biochem. (1983), 131(2), 437-45
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 54 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1982:540565 CAPLUS
DN 97:140565
TI Similarity of acetylcholine receptors of denervated, innervated and embryonic chicken muscles. 1. Molecular species and their purification
AU Sumikawa, Katumi; Mehraban, Fuad; **Dolly, J. Oliver**; Barnard, Eric A.
CS Biochem. Dep., Imp. Coll., London, UK
SO Eur. J. Biochem. (1982), 126(3), 465-72
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 55 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1983:120984 CAPLUS
DN 98:120984
TI Tritiation of .beta.-bungarotoxin and its saturable binding to membranes of cerebrocortical synaptosomes
AU Othman, Iekhsan B.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Biochem. Soc. Trans. (1982), 10(5), 386-7
CODEN: BCSTB5; ISSN: 0300-5127
DT Journal
LA English

L7 ANSWER 56 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1983:29377 CAPLUS

DN 98:29377
TI Preparation of neurotoxic 3H-.beta.-bungarotoxin: demonstration of saturable binding to brain synapses and its inhibition by **toxin** I
AU Othman, Iekhsan B.; Spokes, John W.; **Dolly, J. Oliver**
CS Dep. Biochem., Imp. Coll., London, UK
SO Eur. J. Biochem. (1982), 128(1), 267-76
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 57 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1981:437648 CAPLUS
DN 95:37648
TI Tritiation of .alpha.-bungarotoxin with N-succinimidyl[2,3-3H]propionate. A useful reagent for labeling proteins
AU **Dolly, J. Oliver**; Nockles, Elizabeth A. V.; Lo, Mathew M. S.; Barnard, Eric A.
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, Engl.
SO Biochem. J. (1981), 193(3), 919-23
CODEN: BIJOAK; ISSN: 0306-3275
DT Journal
LA English

L7 ANSWER 58 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1982:129631 CAPLUS
DN 96:129631
TI Production, purification and toxoiding of Clostridium botulinum type A **toxin**
AU Hambleton, Peter; Capel, Brian; Bailey, Nigel; Heron, Nicholas; Crooks, Alan; Melling, Jack; Tse, Chun Kee; **Dolly, J. Oliver**
CS Vaccine Res. Prod. Lab., Cent. Appl. Microbiol. Res., Porton Down/Salisbury/Wilts., UK
SO Biomed. Aspects Botulism, [Proc. Int. Conf.] (1981), 247-60. Editor(s): Lewis George E., Jr. Publisher: Academic, New York, N. Y.
CODEN: 47GRAE
DT Conference
LA English

L7 ANSWER 59 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1981:419970 CAPLUS
DN 95:19970
TI Molecular forms of the acetylcholine receptor from vertebrate muscles and Torpedo electric organ. Interactions with specific ligands
AU Lo, Mathew M. S.; **Dolly, J. Oliver**; Barnard, Eric A.
CS Biochem. Dep., Imp. Coll., London, SW7 2AZ, Engl.
SO Eur. J. Biochem. (1981), 116(1), 155-63
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L7 ANSWER 60 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1982:119590 CAPLUS
DN 96:119590
TI Botulinum neurotoxin type A as a probe for studying neurotransmitter release
AU **Dolly, J. Oliver**; Tse, Chun Kee; Black, J. D.; Williams, R. S.; Wray, D.; Gwilt, M.; Hambleton, Peter; Melling, Jack
CS Dep. Biochem., Imp. Coll., London, SW7 2AZ, UK
SO Biomed. Aspects Botulism, [Proc. Int. Conf.] (1981), 47-64. Editor(s): Lewis, George E., Jr. Publisher: Academic, New York, N. Y.
CODEN: 47GRAE
DT Conference; General Review

LA English

L7 ANSWER 61 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1977:595867 CAPLUS
DN 87:195867
TI Purification and characterization of an acetylcholine receptor from mammalian skeletal muscle
AU **Dolly, J. Oliver**; Barnard, Eric A.
CS Dep. Biochem., Imp. Coll., London, Engl.
SO Biochemistry (1977), 16(23), 5053-60
CODEN: BICHAW
DT Journal
LA English

L7 ANSWER 62 OF 62 CAPLUS COPYRIGHT 1998 ACS
AN 1973:428138 CAPLUS
DN 79:28138
TI Acetylcholine receptor and ion conductance modulator sites at the murine neuromuscular junction. Evidence from specific **toxin** reactions
AU Albuquerque, Edson X.; Barnard, Eric A.; Chiu, Tieh H.; Lapa, Antonio J.; **Dolly, J. Oliver**; Jansson, Sten Erik; Daly, John; Witkop, Bernhard
CS Dep. Pharmacol., State Univ. New York, Buffalo, N. Y., USA
SO Proc. Nat. Acad. Sci. U. S. A. (1973), 70(3), 949-53
CODEN: PNASA6
DT Journal
LA English

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E2	40		AOKI KEI/AU
E3	5	-->	AOKI KEI ROGER/AU
E4	11		AOKI KEIGO/AU
E5	58		AOKI KEIICHI/AU
E6	17		AOKI KEIICHIRO/AU
E7	3		AOKI KEIICHIROU/AU
E8	1		AOKI KEIITIROU/AU
E9	186		AOKI KEIJI/AU
E10	1		AOKI KEIJIRO/AU
E11	10		AOKI KEIKICHI/AU
E12	18		AOKI KEIKO/AU

=> s e3

L12 5 "AOKI KEI ROGER"/AU

=> d bib ab 1-5

L12 ANSWER 1 OF 5 DISSABS COPYRIGHT 1998 UMI Company
AN 82:24870 DISSABS Order Number: AAR8306006
TI EFFECTS OF COLCHICINE ON POLYMORPHONUCLEAR LEUKOCYTES
AU **AOKI, KEI ROGER** [PH.D.]
CS UNIVERSITY OF CALIFORNIA, LOS ANGELES (0031)
SO Dissertation Abstracts International, (1982) Vol. 43, No. 10B, p. 3200. Order No.: AAR8306006. 90 pages.
DT Dissertation
FS DAI
LA English
AB Colchicine, a weak general antiinflammatory agent, has been used to treat acute gouty arthritis for centuries. Although much is known about the subcellular effects of colchicine, its precise mechanism of action for eliciting therapeutic effects in acute gouty

arthritis remains unclear. Since there have been few in vivo studies on the cellular effects of colchicine reported, this investigation was undertaken to determine the effects of colchicine administration on several polymorphonuclear leukocyte (PMN) functions in in vitro (adherence, production/release of a chemotactic factor and phagocytosis) and in vivo (migration). Colchicine administered to rabbits at a non-leukopenic but antiinflammatory dose (0.2 mg/kg, i.v.) was found to: (1) reduce the adhesiveness of peripheral blood PMNs onto nylon fibers via an intrinsic cellular change rather than modification of the cell surface by humoral factors; (2) inhibit the production/release of monosodium urate (MSU) crystal-induced chemotactic factor (CCF) by PMNs; (3) suppress the migration of PMNs induced by MSU crystals or zymosan activated serum (ZAS); (4) have no effect on either the rate or capacity of PMNs in the phagocytosis of yeast. Trimethylcolchicinic acid (TMCA), an analog of colchicine with no antimicrotubular activity, suppressed PMN adhesiveness but was ineffective in suppressing MSU crystal-induced migration in vivo. Oncodazole, an antimicrotubular agent, enhanced the MSU crystal-induced migration of PMNs in vivo. These results suggest that the mechanism(s) which underlies the therapeutic action of colchicine in acute gouty arthritis is likely to be complex. Colchicine interfered with at least three processes that are involved in the migration and accumulation of PMNs at the site of MSU crystal-induced inflammation. Namely, adherence, production/release of CCF and the response of PMNs to complement derived chemotactic factors found in ZAS. The suppression of PMN adherence is probably the least important in view of the results with TMCA treatment. The enhancement of PMN migration of oncodazole suggests that the suppression of PMN migration by colchicine may be unrelated to its effect on the microtubule. The mechanism by which colchicine exerts these effects may be related to its effect on the plasma membrane.

L12 ANSWER 2 OF 5 CAPLUS COPYRIGHT 1998 ACS
AN 1997:640562 CAPLUS
DN 127:298748
TI Injectable therapy with botulinum toxin for control of muscle spasms and pain related to muscle spasms
IN **Aoki, Kei Roger**; Wheeler, Larry A.; Garst, Michael E.
PA Allergan, USA
SO PCT Int. Appl., 55 pp.
CODEN: PIXXD2
PI WO 9734624 A1 970925
DS W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 97-US4643 970320
PRAI US 96-619780 960320
DT Patent
LA English
AB A method for administration of botulinum toxin, includes the steps of (a) selecting at least one neuromuscular blocking agent having a duration of activity shorter than neuromuscular blocking activity of botulinum toxin; (b) selecting at least one muscle of a muscle group; (c) i.m. injecting the selected agent into the selected muscle; (d) observing muscle relaxation in both the selected muscle and other non-selected muscles in the muscle group to det. spill-over, muscle tone and balance; (e) repeating steps (b) - (d) until a final muscle selection is found; and (f) i.m. injecting botulinum toxin into the final muscle selection.

L12 ANSWER 3 OF 5 CAPLUS COPYRIGHT 1998 ACS
AN 1996:102543 CAPLUS
DN 124:127109
TI Conjugates of clostridial toxins and drugs for use in treatment of neuromuscular disorders
IN Dolly, James Oliver; **Aoki, Kei Roger**; Wheeler, Larry Allen; Garst, Michael Elwood
PA Allergan, Inc., USA
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
PI WO 9532738 A1 951207
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-GB1253 950531
PRAI GB 94-10870 940531
GB 94-10871 940531
DT Patent
LA English
AB A chem. conjugate for treating a nerve cell related disorder is provided. This conjugate includes an active or inactive Clostridial toxin having specificity for a target nerve cell. The toxin is conjugated to a drug or other bioactive mol. without affecting the toxin's ability to enter the target nerve cell. Recombinant Ala-234 tetanus toxin L chain mutant was prepd. and a reconstituted tetanus toxin dimer prepd. with the L chain mutant and native H chain was shown to be nontoxic. The process of conjugating vesamicol to this reconstituted, inactive toxin was described. Mutant botulinum toxin A L chains were also prepd. and the reconstituted dimer toxin shown to be inactive.

L12 ANSWER 4 OF 5 CAPLUS COPYRIGHT 1998 ACS
AN 1992:46309 CAPLUS
DN 116:46309
TI Ophthalmic compositions containing bufroline or its derivatives
IN **Aoki, Kei Roger**; Wheeler, Larry A.
PA Allergan, Inc., USA
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
PI WO 9112004 A1 910822
DS W: AU, BB, BG, BR, CA, FI, HU, JP, KP, KR, LK, MC, MG, MW, NO, PL, RO, SD, SU
RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG
AI WO 91-US805 910206
PRAI US 90-476834 900207
US 91-646667 910128
DT Patent
LA English
OS MARPAT 116:46309
AB Ophthalmic compns. contg. 0.1-6.0% (wt./vol.) 1,7-phenanthroline-2,8-dicarboxylic acid derivs. [I; R1, R2 = OH, (un)substituted amino, OR3; R3 = (un)substituted aliph. hydrocarbyl] or their salts are topically administered for the prevention or treatment of inflammatory conditions initiated by an immune response. Thus, a passive ocular anaphylaxis reaction was elicited in the eyelid of rats by IgE and bufrolin solns. in an artificial tear were administered to the eyes 1 min prior to the challenge with an i.v. soln. of antigen and Evans blue. A low quantity of Evans blue in the tissue represented a redn. in vasoactive mediator release. Ophthalmic formulations in the form of a soln., gel, emulsion, and ointment are given.

L12 ANSWER 5 OF 5 CAPLUS COPYRIGHT 1998 ACS
AN 1983:447659 CAPLUS
DN 99:47659
TI Effects of colchicine on polymorphonuclear leukocytes
AU **Aoki, Kei Roger**
CS Univ. California, Los Angeles, CA, USA
SO (1982) 90 pp. Avail.: Univ. Microfilms Int., Order No. DA8306006
From: Diss. Abstr. Int. B 1983, 43(10), 3200
DT Dissertation
LA English
AB Unavailable

=> e wheeler larry allen/au

E1	13	WHEELER LARRY/AU
E2	33	WHEELER LARRY A/AU
E3	2 -->	WHEELER LARRY ALLEN/AU
E4	1	WHEELER LARRY EUGENE/AU
E5	1	WHEELER LARRY JAMES/AU
E6	4	WHEELER LARRY M/AU
E7	3	WHEELER LARRY O/AU
E8	2	WHEELER LARRY OWEN/AU
E9	1	WHEELER LAURA ALLISON/AU
E10	1	WHEELER LAURA MAUDE/AU
E11	1	WHEELER LAVERNE C/AU
E12	3	WHEELER LAWRENCE A/AU

=> s e2 or e3

L13 35 "WHEELER LARRY A"/AU OR "WHEELER LARRY ALLEN"/AU

=> s l13 and neurotoxin

L14 0 L13 AND NEUROTOXIN

=> s l13 and cellubrevin

L15 0 L13 AND CELLUBREVIN

=> s l13 and toxin

L16 2 L13 AND TOXIN

=> d bib ab 1-2

L16 ANSWER 1 OF 2 CAPLUS COPYRIGHT 1998 ACS
AN 1997:640562 CAPLUS
DN 127:298748
TI Injectable therapy with botulinum **toxin** for control of muscle spasms and pain related to muscle spasms
IN Aoki, Kei Roger; **Wheeler, Larry A.**; Garst, Michael E.
PA Allergan, USA
SO PCT Int. Appl., 55 pp.
CODEN: PIXXD2
PI WO 9734624 A1 970925
DS W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 97-US4643 970320

PRAI US 96-619780 960320
DT Patent
LA English
AB A method for administration of botulinum **toxin**, includes the steps of (a) selecting at least one neuromuscular blocking agent having a duration of activity shorter than neuromuscular blocking activity of botulinum **toxin**; (b) selecting at least one muscle of a muscle group; (c) i.m. injecting the selected agent into the selected muscle; (d) observing muscle relaxation in both the selected muscle and other non-selected muscles in the muscle group to det. spill-over, muscle tone and balance; (e) repeating steps (b) - (d) until a final muscle selection is found; and (f) i.m. injecting botulinum **toxin** into the final muscle selection.

L16 ANSWER 2 OF 2 CAPLUS COPYRIGHT 1998 ACS
AN 1996:102543 CAPLUS
DN 124:127109
TI Conjugates of clostridial toxins and drugs for use in treatment of neuromuscular disorders
IN Dolly, James Oliver; Aoki, Kei Roger; **Wheeler, Larry Allen** ; Garst, Michael Elwood
PA Allergan, Inc., USA
SO PCT Int. Appl., 67 pp.
CODEN: PIXXD2
PI WO 9532738 A1 951207
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-GB1253 950531
PRAI GB 94-10870 940531
GB 94-10871 940531
DT Patent
LA English
AB A chem. conjugate for treating a nerve cell related disorder is provided. This conjugate includes an active or inactive Clostridial **toxin** having specificity for a target nerve cell. The **toxin** is conjugated to a drug or other bioactive mol. without affecting the **toxin**'s ability to enter the target nerve cell. Recombinant Ala-234 tetanus **toxin** L chain mutant was prepd. and a reconstituted tetanus **toxin** dimer prepd. with the L chain mutant and native H chain was shown to be nontoxic. The process of conjugating vesamicol to this reconstituted, inactive **toxin** was described. Mutant botulinum **toxin** A L chains were also prepd. and the reconstituted dimer **toxin** shown to be inactive.

=> s l13 and vamp

L17 0 L13 AND VAMP

=> e garst michael elwood/au

E1 1 GARST MICHAEL/AU
E2 79 GARST MICHAEL E/AU
E3 2 --> GARST MICHAEL ELWOOD/AU
E4 6 GARST P/AU
E5 10 GARST P D/AU
E6 1 GARST PETER FREEDMAN/AU
E7 1 GARST R/AU
E8 1 GARST R D/AU
E9 1 GARST R G/AU
E10 7 GARST R H/AU

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E11          10     GARST R J/AU
E12           1     GARST ROGER/AU

=> s e2 or e3

L18          81 "GARST MICHAEL E"/AU OR "GARST MICHAEL ELWOOD"/AU

=> s l18 and toxin

L19           2 L18 AND TOXIN

=> d bib ab 1-2


L19  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 1998 ACS
AN   1997:640562  CAPLUS
DN   127:298748
TI   Injectable therapy with botulinum toxin for control of
     muscle spasms and pain related to muscle spasms
IN   Aoki, Kei Roger; Wheeler, Larry A.; Garst, Michael E.
PA   Allergan, USA
SO   PCT Int. Appl., 55 pp.
     CODEN: PIXXD2
PI   WO 9734624 A1  970925
DS   W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
         DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC,
         LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
         RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM,
         AZ, BY, KG, KZ, MD, RU, TJ, TM
     RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB,
         GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI   WO 97-US4643  970320
PRAI US 96-619780  960320
DT   Patent
LA   English
AB   A method for administration of botulinum toxin, includes
     the steps of (a) selecting at least one neuromuscular blocking agent
     having a duration of activity shorter than neuromuscular blocking
     activity of botulinum toxin; (b) selecting at least one
     muscle of a muscle group; (c) i.m. injecting the selected agent into
     the selected muscle; (d) observing muscle relaxation in both the
     selected muscle and other non-selected muscles in the muscle group
     to det. spill-over, muscle tone and balance; (e) repeating steps (b)
     - (d) until a final muscle selection is found; and (f) i.m.
     injecting botulinum toxin into the final muscle selection.

L19  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 1998 ACS
AN   1996:102543  CAPLUS
DN   124:127109
TI   Conjugates of clostridial toxins and drugs for use in treatment of
     neuromuscular disorders
IN   Dolly, James Oliver; Aoki, Kei Roger; Wheeler, Larry Allen;
     Garst, Michael Elwood
PA   Allergan, Inc., USA
SO   PCT Int. Appl., 67 pp.
     CODEN: PIXXD2
PI   WO 9532738 A1  951207
DS   W:  AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI,
         GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD,
         MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ,
         TM, TT
     RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR,
         IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI   WO 95-GB1253  950531
PRAI GB 94-10870  940531
```

GB 94-10871 940531
DT Patent
LA English
AB A chem. conjugate for treating a nerve cell related disorder is provided. This conjugate includes an active or inactive Clostridial **toxin** having specificity for a target nerve cell. The **toxin** is conjugated to a drug or other bioactive mol. without affecting the **toxin**'s ability to enter the target nerve cell. Recombinant Ala-234 tetanus **toxin** L chain mutant was prepd. and a reconstituted tetanus **toxin** dimer prepd. with the L chain mutant and native H chain was shown to be nontoxic. The process of conjugating vesamicol to this reconstituted, inactive **toxin** was described. Mutant botulinum **toxin** A L chains were also prepd. and the reconstituted dimer **toxin** shown to be inactive.

=> s l18 and cellubrevin

L20 0 L18 AND CELLUBREVIN

=> s vamp and synaptobrevin

L21 477 VAMP AND SYNAPTOBREVIN

=> s l21 and toxin

L22 192 L21 AND TOXIN

=> s l22 and botulin?

L23 128 L22 AND BOTULIN?

=> dup rem l23

PROCESSING COMPLETED FOR L23
L24 54 DUP REM L23 (74 DUPLICATES REMOVED)

=> d bib ab 1-53

L24 ANSWER 1 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1998:35261 CAPLUS
DN 128:111750
TI H+ secretion is inhibited by clostridial toxins in an inner medullary collecting duct cell line
AU Alexander, Edward A.; Shih, Theodora; Schwartz, John H.
CS Renal Section, Boston Medical Center, Departments Medicine, Physiology, Pathology, Boston University School Medicine, Boston, MA, 02118-2908, USA
SO Am. J. Physiol. (1997), 273(6, Pt. 2), F1054-F1057
CODEN: AJPHAP; ISSN: 0002-9513
PB American Physiological Society
DT Journal
LA English
AB Renal epithelial cell H+ secretion is an exocytic-endocytic phenomenon. In the inner medullary collecting duct (IMCD) cell line, which we have utilized as a model of renal epithelial cell acid secretion, we found previously that acidification increased exocytosis and alkalinization increased endocytosis. It is likely, therefore, that the rate of proton secretion is regulated by the membrane insertion and retrieval of proton pumps. There is abundant evidence from studies in the nerve terminal and the chromaffin cell that vesicle docking, membrane fusion, and discharge of vesicular contents (exocytosis) involve a series of interactions among so-called trafficking proteins. The clostridial toxins,

botulinum and tetanus, are proteases that specifically inactivate some of these proteins. In these expts. we demonstrated, by immunoblot and immunopptn., the presence in this IMCD cell line of the specific protein targets of these toxins, **synaptobrevin**/vesicle-assocd. membrane proteins (**VAMP**), syntaxin, and synaptosomal-assocd. protein-25 (SNAP-25). Furthermore, we showed that these toxins markedly inhibit the capacity of these cells to realkalinize after an acid load. Thus these data provide new insight into the mechanism for H+ secretion in the IMCD.

L24 ANSWER 2 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 1
AN 97296910 EMBASE
TI Ca2+ or Sr2+ partially rescues synaptic transmission in hippocampal cultures treated with **botulinum toxin** A and C, but not tetanus **toxin**.
AU Capogna M.; McKinney R.A.; O'Connor V.; Gahwiler B.H.; Thompson S.M.
CS Dr. M. Capogna, Brain Research Institute, University of Zurich, August Forel-Strasse 1, CH-8029 Zurich, Switzerland
SO Journal of Neuroscience, (1997) 17/19 (7190-7202).
Refs: 67
ISSN: 0270-6474 CODEN: JNRSDS
CY United States
DT Journal
FS 002 Physiology
004 Microbiology
008 Neurology and Neurosurgery
LA English
SL English
AB **Botulinum** (BoNT/A-G) and tetanus toxins (TeNT) are zinc endopeptidases that cleave proteins associated with presynaptic terminals (SNAP-25, syntaxin, or **VAMP**/**synaptobrevin**) and block neurotransmitter release. Treatment of hippocampal slice cultures with BoNT/A, BoNT/C, BoNT/E, or TeNT prevented the occurrence of spontaneous or miniature EPSCs (sEPSCs or mEPSCs) as well as the [Ca2+](o)-independent increase in their frequency induced by phorbol ester, 0.5 nM .alpha.-latrotoxin, or sucrose. [Ca2+](o)-independent and -dependent release thus requires that the target proteins of clostridial neurotoxins be uncleaved. In contrast, significant increases in mEPSC frequency were produced in BoNT-treated, but not TeNT-treated, cultures by application of the Ca2+ ionophore ionomycin in the presence of 10 mM [Ca2+](o). The frequency of sEPSCs was increased in BeNT-treated, but not TeNT-treated, cultures by increasing [Ca2+](o) from 2.8 to 5-10 mM or by applying 5 mM Sr2+. Large Ca2+ and Sr2+ influxes thus can rescue release after BeNT treatment, albeit less than in control cultures. The nature of the **toxin**-induced modification of Ca2+-dependent release was assessed by recordings from monosynaptically coupled CA3 cell pairs. The paired-pulse ratio of unitary EPSCs evoked by two presynaptic action potentials in close succession was 0.5 in control cultures, but it was 1.4 and 1.2 in BoNT/A- or BoNT/C-treated cultures when recorded in 10 mM [Ca(2)+](o). Log-log plots of unitary EPSC amplitude versus [Ca(2)+](o) were shifted toward higher [Ca(2)+](o) in BoNT/A- or BoNT/C-treated cultures, but their slope was unchanged and the maximal EPSC amplitudes were reduced. We conclude that BoNTs reduce the Ca2+ sensitivity of the exocytotic machinery and the number of quanta released.

L24 ANSWER 3 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 2
AN 1998007230 EMBASE
TI Functional importance of **synaptobrevin** and SNAP-25 during exocytosis of histamine by rat gastric enterochromaffin-like cells.
AU Hohne-Zell B.; Galler A.; Schepp W.; Gratzl M.; Prinz C.
CS Dr. M. Gratzl, Anatomisches Institut, Technischen Universitat

Munchen, Biedersteiner Strasse 29, 80802 Munich, Germany.
gratzl@lrz.tu-muenchen.de
SO Endocrinology, (1997) 138/12 (5518-5526).
Refs: 52
ISSN: 0013-7227 CODEN: ENDOAO
CY United States
DT Journal; Article
FS 003 Endocrinology
LA English
SL English
AB Gastric enterochromaffin-like (ECL) cells release histamine upon stimulation with gastrin in a calcium-dependent manner. The intracellular mechanisms and proteins mediating exocytosis of histamine-containing vesicles in ECL cells have not been determined yet. We used immunocytochemistry to show the localization of SNAP-25 (synaptosome-associated protein of 25 kDa) and **synaptobrevin** **VAMP** (vesicle-associated membrane protein) in ECL cells of the rat gastric mucosa and in isolated, highly enriched ECL cells, which were identified with an antibody directed against the marker enzyme histidine decarboxylase. Immunoblots of isolated ECL cells demonstrated the presence of SNAP-25, **synaptobrevin**, synaptophysin, synaptotagmin, and syntaxin. Histamine release from isolated ECL cells permeabilized with 8 .mu.M digitonin (2 min) was stimulated approximately 2.5-fold upon exposure to calcium (30 .mu.M; 10- min incubation). Preincubation with 1 .mu.M tetanus **toxin** light chain for 15 min attenuated calcium-induced histamine release by 40-50% and almost completely cleaved **synaptobrevin**. **Botulinum** neurotoxin A (100 nM) totally blocked calcium-induced histamine release and cleaved SNAP-25. We conclude that **synaptobrevin**, synaptophysin, synaptotagmin, SNAP-25, and syntaxin are present in gastric ECL cells. Inhibition of histamine secretion by clostridial neurotoxins associated with the cleavage of **synaptobrevin** and SNAP-25 implicates the functional importance of these proteins in the docking and fusion of histamine vesicles.

L24 ANSWER 4 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 3
AN 97046506 EMBASE
TI Cooperative exosite-dependent cleavage of **synaptobrevin** by tetanus **toxin** light chain.
AU Cornille F.; Martin L.; Lenoir C.; Cussac D.; Roques B.P.; Fournie-Zaluski M.-C.
CS B.P. Roques, U266 INSERM, URA D1500 CNRS, Universite Rene Descartes, 4, Avenue de l'Observatoire, 75270 Paris Cedex 06, France
SO Journal of Biological Chemistry, (1997) 272/6 (3459-3464).
Refs: 42
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB The light chain (L chain) of tetanus neurotoxin (TeNT) has been shown to have been endowed with zinc endopeptidase activity, selectively directed toward the Gln76-Phe77 bond of **synaptobrevin**, a vesicle-associated membrane protein (**VAMP**) critically involved in neuroexocytosis. In previous reports, truncations at the NH2 and COOH terminus of **synaptobrevin** have shown that the sequence 39-88 of **synaptobrevin** is the minimum substrate of TENT, suggesting either the requirement of a well defined three-dimensional structure of **synaptobrevin** or a role in the mechanism of substrate hydrolysis for residues distal from the cleavage site. In this study, the addition of NH2- and COOH-terminal peptides of **synaptobrevin**, S 27-55 (S1) and S 82-93 (S2), to the

synaptobrevin fragment S 56-81 allowed the cleavage of this latter peptide by TeNT to occur. This appears to result from an activation process mediated by the simultaneous binding of S1 and S2 with complementary sites present on TeNT as shown by surface plasmon resonance experiments and the determination of kinetic constants. All these results favor an exosite-controlled hydrolysis of **synaptobrevin** by TeNT, probably involving a conformational change of the **toxin**. This could account for the high degree of substrate specificity of TeNT and, probably, **botulinum** neurotoxins.

L24 ANSWER 5 OF 54 MEDLINE DUPLICATE 4
AN 1998097985 MEDLINE
DN 98097985
TI H+ secretion is inhibited by clostridial toxins in an inner medullary collecting duct cell line.
AU Alexander E A; Shih T; Schwartz J H
CS Renal Section, Boston Medical Center, Massachusetts, USA.
NC DK-28164 (NIDDK)
SO AMERICAN JOURNAL OF PHYSIOLOGY, (1997 Dec) 273 (6 Pt 2) F1054-7. Journal code: 3U8. ISSN: 0002-9513.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199804
EW 19980402
AB Renal epithelial cell H+ secretion is an exocytic-endocytic phenomenon. In the inner medullary collecting duct (IMCD) cell line, which we have utilized as a model of renal epithelial cell acid secretion, we found previously that acidification increased exocytosis and alkalinization increased endocytosis. It is likely, therefore, that the rate of proton secretion is regulated by the membrane insertion and retrieval of proton pumps. There is abundant evidence from studies in the nerve terminal and the chromaffin cell that vesicle docking, membrane fusion, and discharge of vesicular contents (exocytosis) involve a series of interactions among so-called trafficking proteins. The clostridial toxins, **botulinum** and tetanus are proteases that specifically inactivate some of these proteins. In these experiments we demonstrated, by immunoblot and immunoprecipitation, the presence in this IMCD cell line of the specific protein targets of these toxins, **synaptobrevin**/vesicle-associated membrane proteins (**VAMP**), syntaxin, and synaptosomal-associated protein-25 (SNAP-25). Furthermore, we showed that these toxins markedly inhibit the capacity of these cells to realkalinize after an acid load. Thus these data provide new insight into the mechanism for H+ secretion in the IMCD.

L24 ANSWER 6 OF 54 MEDLINE DUPLICATE 5
AN 97441748 MEDLINE
DN 97441748
TI [Action mechanisms of **botulinum** neurotoxins and tetanus neurotoxins].
Mecanismes d'action des neurotoxines botuliques et de la neurotoxine tetanique.
AU Deloye F; Doussau F; Poulain B
CS Laboratoire de Neurobiologie Cellulaire et Moleculaire, UPR 9040 du CNRS, Gif-sur-Yvette.
SO COMPTES RENDUS DES SEANCES DE LA SOCIETE DE BIOLOGIE ET DE SES FILIALES, (1997) 191 (3) 433-50. Ref: 85
Journal code: CA2. ISSN: 0037-9026.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, TUTORIAL)
LA French
FS Priority Journals
EM 199712
EW 19971201
AB Tetanus (TeNT) neurotoxin and **botulinum** (BoNT, serotypes A-G) neurotoxins are di-chain bacterial proteins of MW-150 kDa which are also termed as clostridial neurotoxins. They are the only causative agents of two severe neuroparalytic diseases, namely tetanus and botulism. The peripheral muscle spasms which characterise tetanus are due to a blockade of inhibitory (GABAergic and glycinergic) synapses in the central nervous system leading to a motor neurones desinhibition. In contrast, botulism symptoms are only peripheral. They are consequent to a near irreversible and highly selective inhibition of acetyl-choline release at the motor nerve endings innervating skeletal muscles. During the past decade, the cellular and molecular modes of action of clostridial neurotoxins has been near completely elucidated. After a binding step of the neurotoxins to specific membrane acceptors located only on nerve terminals, BoNTs and TeNT are internalized into neurons. Inside their target neurones, the intracellularly active moiety (their light chain) is translocated from the endosomal compartment to the cytosol. The neurotoxins' light chains are zinc-dependent (endopeptidases which are specific for one among three synaptic proteins (**VAMP/synaptobrevin**, syntaxin or SNAP-25) implicated in neurotransmitter exocytosis. The presence of distinct targets for BoNTs and TeNT correlates well with the observed quantal alterations of neurotransmitter release which characterize certain **toxin** serotypes. In addition, evidence for a second, non-proteolytic, inhibitory mechanism of action has been provided recently. Most likely, this additional blocking action involves the activation of neurone transglutaminases. Due to their specific action on key proteins of the exocytosis apparatus, clostridial neurotoxins are now widely used as molecular tools to study exocytosis.

L24 ANSWER 7 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 6
AN 97203184 EMBASE
TI The interaction of synaptic vesicle-associated membrane protein/ **synaptobrevin** with **botulinum** neurotoxins D and F.
AU Pellizzari R.; Mason S.; Shone C.C.; Montecucco C.
CS C. Montecucco, Centro CNR Biomembrane, Dipartemento di Scienze Biomediche, Universita di Padova, Via G. Colombo 3, 35100 Padova, Italy
SO FEBS Letters, (1997) 409/3 (339-342).
Refs: 36
ISSN: 0014-5793 CODEN: FEBLAL
PUI S 0014-5793(97)00482-1
CY Netherlands
DT Journal
FS 004 Microbiology
052 Toxicology
LA English
SL English
AB **Botulinum** neurotoxins type D and F are zinc-endopeptidases with a unique specificity for **VAMP/synaptobrevin**, an essential component of the exocytosis apparatus. **VAMP** contains two copies of a nine residue motif, termed V1 and V2, which are determinants of the interaction with tetanus and **botulinum** B and G neurotoxins. Here, we show that V1 plays a major role in **VAMP** recognition by **botulinum** neurotoxins D and P and that V2 is also involved in F binding. Site-directed mutagenesis of V1 and V2 indicates that different residues are the determinants of the **VAMP** interaction with the two endopeptidases. The study of the **VAMP**-neurotoxins

interaction suggest a pairing of the V1 and V2 segments.

L24 ANSWER 8 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97162984 EMBASE
TI Functional studies in 3T3L1 cells support a role for SNARE proteins in insulin stimulation of GLUT4 translocation.
AU MacAulay S.L.; Hewish D.R.; Gough K.H.; Stoichevska V.; MacPherson S.F.; Jagadish M.; Ward C.W.
CS S.L. MacAulay, CSIRO, Division of Biomolecular Engineering, 343 Royal Parade, Parkville, Vic. 3052, Australia
SO Biochemical Journal, (1997) 324/1 (217-224).
Refs: 53
ISSN: 0264-6021 CODEN: BIJOAK
CY United Kingdom
DT Journal
FS 003 Endocrinology
029 Clinical Biochemistry
LA English
SL English
AB Insulin stimulation of glucose transport in the major insulin-responsive tissues results predominantly from the translocation to the cell surface of a particular glucose transporter isoform, GLUT4, residing normally under basal conditions in intracellular vesicular structures. Recent studies have identified the presence of vesicle-associated membrane protein (**VAMP**) 2, a protein involved in vesicular trafficking in secretory cell types, in the vesicles of insulin-sensitive cells that contain GLUT4. The plasma membranes of insulin-responsive cells have also been shown to contain syntaxin 4 and the 25 kDa synaptosome-associated protein (SNAP-25), two proteins that form a complex with **VAMP** 2. The potential functional involvement of **VAMP** 2, SNAP-25 and syntaxin 4 in the trafficking of GLUT4 was assessed in the present study by determining the effect on GLUT4 translocation of microinjection of toxins that specifically cleave VAMPs or SNAP-25, or microinjection of specific peptides from **VAMP** 2 and syntaxin 4. Microinjection of tetanus **toxin** light chain or **botulinum** D **toxin** light chain resulted in an 80 and 61% inhibition respectively of insulin stimulation of GLUT4 translocation in 3T3L1 cells assessed using the plasma-membrane lawn assay. **Botulinum** A **toxin** light chain, which cleaves SNAP-25, was without effect. Microinjection of an N-terminal **VAMP** 2 peptide (residues 1-26) inhibited insulin stimulation of GLUT4 translocation by 54%. A syntaxin 4 peptide (residues 106-122) inhibited insulin stimulation of GLUT4 translocation by 40% whereas a syntaxin 1c peptide (residues 226-260) was without effect. These data taken together strongly suggest a role for **VAMP** 2 in GLUT4 trafficking and also for syntaxin 4. They further indicate that the isoforms of SNAP-25 isolated to date that are sensitive to cleavage by **botulinum** A **toxin** light chain do not appear to be involved in GLUT4 translocation.

L24 ANSWER 9 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 7
AN 97171428 EMBASE
TI Cleavage of the **synaptobrevin**/vesicle-associated membrane protein (**VAMP**) of the mouse brain by the recombinant light chain of Clostridium **botulinum** type B **toxin**.
AU Rhee S.D.; Jung H.H.; Yang G.-H.; Moon Y.S.; Yang K.-H.
CS K.-H. Yang, Department of Biological Sciences, KAIST, Taejon, Korea, Republic of. khyang@sorak.kaist.ac.kr
SO FEMS Microbiology Letters, (1997) 150/2 (203-208).
Refs: 21
ISSN: 0378-1097 CODEN: FMLED7
PUI S 0378-1097(97)00114-6
CY Netherlands

DT Journal
FS 004 Microbiology
LA English
SL English
AB The light chain of Clostridium **botulinum** type B **toxin** was expressed in Escherichia coli using the expression vector pET-3a containing phage T7 promoter. The expressed protein was then purified by DEAE-cellulose and phosphocellulose chromatography and the proteolytic activity of the purified light chain was studied. The purified recombinant light chain cleaved **synaptobrevin** when mixed with the mouse brain microsome and the proteolytic activity of the light chain was inhibited if a metal chelating agent such as EDTA or 2,2'-dipyridyl was added. The recombinant light chain cleaved **synaptobrevin** more effectively than the native type B **toxin**. When the native **toxin** was trypsinized and was reduced with DTT, its proteolytic activity was similar to that of the recombinant light chain.

L24 ANSWER 10 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1997:519072 CAPLUS
DN 127:132016
TI **VAMP**-specific **botulinum** neurotoxins
AU Schiavo, Giampietro
CS Imperial Cancer Research Foundation, London, WC2A 3PX, UK
SO Guideb. Protein Toxins Their Use Cell Biol. (1997), 103-105. Editor(s): Rappuoli, Rino; Montecucco, Cesare. Publisher: Oxford University Press, Oxford, UK.
CODEN: 64UWAW
DT Conference; General Review
LA English
AB A review and discussion with 24 refs. **Botulinum** neurotoxins are a group of closely related protein toxins (seven different serotypes A-G) produced by different bacterial strains of the genus Clostridium. All of them show abs. tropism for the neuromuscular junction, where they bind still unidentified receptors in a strictly serotype specific manner. This binding step is followed by the entry of the **toxin** into the cytoplasm of the motor neurons and by specific proteolytic cleavage of intracellular targets. Four out of seven serotypes of **botulinum** neurotoxins cleave **VAMP**/**synaptobrevin**, a protein of small synaptic vesicles. This results in a loss of function of the neuroexocytosis machinery and thus a blockade of transmitter release.

L24 ANSWER 11 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 8
AN 96260202 EMBASE
TI Structural determinants of the specificity for synaptic vesicle-associated membrane protein/**synaptobrevin** of tetanus and **botulinum** type B and G neurotoxins.
AU Pellizzari R.; Rossetto O.; Lozzi L.; Giovedi S.; Johnson E.; Shone C.C.; Montecucco C.
CS Dipartimento di Scienze Biomediche, Via Trieste 75, 35100 Padova, Italy
SO Journal of Biological Chemistry, (1996) 271/34 (20353-20358). ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB Tetanus and **botulinum** neurotoxins type B and G are zinc-endopeptidases of remarkable specificity. They recognize and

cleave a synaptic vesicle- associated membrane protein (**VAMP**)/**synaptobrevin**, an essential protein component of the vesicle docking and fusion apparatus. **VAMP** contains two copies of a nine-residue motif, also present in SNAP-25 (synaptosomal- associated protein of 25 kDa) and syntaxin, the two other substrates of clostridial neurotoxins. This motif was suggested to be a determinant of the target specificity of neurotoxins. Antibodies raised against this motif cross-react among **VAMP**, SNAP-25, and syntaxin and inhibit the proteolytic activity of the neurotoxins. Moreover, the various neurotoxins cross-inhibit each other's proteolytic action. The role of the three negatively charged residues of the motif in neurotoxin recognition was probed by site-directed mutagenesis. Substitution of acidic residues in both copies of the **VAMP** motif indicate that the first one is involved in tetanus neurotoxin recognition, whereas the second one is implicated in binding **botulinum** B and G neurotoxins. These results suggest that the two copies of the motif have a tandem association in the **VAMP** molecule.

L24 ANSWER 12 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97009114 EMBASE
TI Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins.
AU Cheatham B.; Volchuk A.; Kahn C.R.; Wang L.; Rhodes C.J.; Klip A.
CS United States. cheathab@joslab.harvard.edu
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) 93/26 (15169-15173).
Refs: 32
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB A major physiological role of insulin is the regulation of glucose uptake into skeletal and cardiac muscle and adipose tissue, mediated by an insulin-stimulated translocation of GLUT4 glucose transporters from an intracellular vesicular pool to the plasma membrane. This process is similar to the regulated docking and fusion of vesicles in neuroendocrine cells, a process that involves SNARE-complex proteins. Recently, several SNARE proteins were found in adipocytes: vesicle-associated membrane protein (**VAMP**- 2), its related homologue cellubrevin, and syntaxin-4. In this report we show that treatment of permeabilized 3T3-L1 adipocytes with **botulinum** neurotoxin D, which selectively cleaves **VAMP**-2 and cellubrevin, inhibited the ability of insulin to stimulate translocation of GLUT4 vesicles to the plasma membrane. Furthermore, treatment of the permeabilized adipocytes with glutathione S-transferase fusion proteins encoding soluble forms of **VAMP** -2 or syntaxin-4 also effectively blocked insulin-regulated GLUT4 translocation. These results provide evidence of a functional role for SNARE-complex proteins in insulin- stimulated glucose uptake and suggest that adipocytes utilize a mechanism of regulating vesicle docking and fusion analogous to that found in neuroendocrine tissues.

L24 ANSWER 13 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 9
AN 97020992 EMBASE
TI Evidence for a functional link between Rab3 and the SNARE complex.
AU Johannes L.; Doussau F.; Clabecq A.; Henry J.-P.; Darchen F.; Poulain B.
CS F. Darchen, Ctr. National Recherche Scientifique, UPR 9071, Institut Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France. darchen@ibpc.fr

SO Journal of Cell Science, (1996) 109/12 (2875-2884).
Refs: 53
ISSN: 0021-9533 CODEN: JNCSAI
CY United Kingdom
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB Rab3 is a monomeric GTP-binding protein associated with secretory vesicles which has been implicated in the control of regulated exocytosis. We have exploited Rab3 mutant proteins to investigate the function of Rab3 in the process of neurotransmitter release from Aplysia neurons. A GTPase-deficient Rab3 mutant protein was found to inhibit acetylcholine release suggesting that GTP hydrolysis by Rab3 is rate-limiting in the exocytosis process. This effect was abolished by a mutation in the effector domain, and required the association of Rab3 with membranes. In order to determine the step at which Rab3 interferes with the secretory process, tetanus and **botulinum** type A neurotoxins were applied to Aplysia neurons pre-injected with the GTPase-deficient Rab3 mutant protein. These neurotoxins are Zn2+-dependent proteases that cleave **VAMP/synaptobrevin** and SNAP-25, two proteins which can form a ternary complex (termed the SNARE complex) with syntaxin and have been implicated in the docking of synaptic vesicles at the plasma membrane. The onset of **toxin**-induced inhibition of neurotransmitter release was strongly delayed in these cells, indicating that the mutant Rab3 protein led to the accumulation of a **toxin**-insensitive component of release. Since tetanus and **botulinum** type A neurotoxins cannot attack their targets, **VAMP/synaptobrevin** and SNAP-25, when the latter are engaged in the SNARE complex, we propose that Rab3 modulates the activity of the fusion machinery by controlling the formation or the stability of the SNARE complex.

L24 ANSWER 14 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 10
AN 96217163 EMBASE
TI Development of novel assays for **botulinum** type A and B neurotoxins based on their endopeptidase activities.
AU Hallis B.; James B.A.F.; Shone C.C.
CS Protein Toxins Section, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom
SO Journal of Clinical Microbiology, (1996) 34/8 (1934-1938).
ISSN: 0095-1137 CODEN: JCMIDW
CY United States
DT Journal
FS 004 Microbiology
LA English
SL English
AB A novel assay method based on the endopeptidase activities of the **botulinum** neurotoxins has been developed and applied to the detection of **botulinum** type A and B toxins. An assay system developed for the detection of **botulinum** type B neurotoxin (BoNT/B) is based on the cleavage of a synthetic peptide substrate representing amino acid residues 60 to 94 of the intracellular target protein for the **toxin**, **VAMP** (vesicle-associated membrane protein, or **synaptobrevin**). In this assay system, immobilized **VAMP** (60-94) peptide substrate is cleaved by BoNT/B at the Gln-76-Phe-77 bond, leaving the C-terminal cleavage fragment on the solid phase. This fragment is then detected by the addition of an antibody-enzyme reagent which specifically recognizes the newly exposed N terminus of the cleavage product. The developed assay was specific to BoNT/B, showing no cross-reactivity with other clostridial neurotoxins, and had a

sensitivity for BoNT/B of 0.6 to 4.5 ng/ml, which could be increased to 0.1 to 0.2 ng/ml by using an assay amplification system based on catalyzed reporter deposition. Trypsin treatment of BoNT/B samples, which converts the single-chain **toxin** to the active di-chain form, was found to increase the sensitivity of the endopeptidase assay from 5- to 10-fold. An endopeptidase assay for BoNT/A, based on the cleavage of a peptide substrate derived from the protein SNAP- 25 (synaptosome-associated protein), was also developed and characterized.

L24 ANSWER 15 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 96208268 EMBASE
TI Nitric oxide modulates synaptic vesicle docking/fusion reactions.
AU Meffert M.K.; Calakos N.C.; Scheller R.H.; Schulman H.
CS Department of Neurobiology, Howard Hughes Medical Institute, Stanford University Sch. of Medicine, Stanford, CA 94305, United States
SO Neuron, (1996) 16/6 (1229-1236).
ISSN: 0896-6273 CODEN: NERNET
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
LA English
SL English
AB Nitric oxide (NO) stimulates calcium-independent neurotransmitter release from synaptosomes. NO-stimulated release was found to be inhibited by **Botulinum** neurotoxins that inactivate the core complex of synaptic proteins involved in the docking and fusion of synaptic vesicles. In experiments using recombinant proteins, NO donors increased formation of the **VAMP**/SNAP- 25/syntaxin 1a core complex and inhibited the binding of n-sec1 to syntaxin 1a. The combined effects of these activities is predicted to promote vesicle docking/fusion. The sulfhydryl reagent NEM inhibited the binding of n-sec1 to syntaxin 1a, while .beta.-ME could reverse the NO-enhanced association of **VAMP**/SNAP-25/syntaxin 1a. These data suggest that post-translational modification of sulfhydryl groups by a nitrogen monoxide (likely to be NO+) alters the synaptic protein interactions that regulate neurotransmitter release and synaptic plasticity.

L24 ANSWER 16 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97016472 EMBASE
TI Bacterial protein toxins and cell vesicle trafficking.
AU Montecucco C.; Papini E.; Schiavo G.
CS C. Montecucco, Centro CNR Biomembrane, Dipartimento di Scienze Biomediche, Universita di Padova, Via Trieste 75, I-35121 Padova, Italy. toxin@cribi1.bio.unipd.it
SO Experientia, (1996) 52/12 (1026-1032).
Refs: 91
ISSN: 0014-4754 CODEN: EXPEAM
CY Switzerland
DT Journal
FS 004 Microbiology
029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB A group of bacterial protein toxins interfere with vesicular trafficking inside cells. Clostridial neurotoxins affect mainly the highly regulated fusion of neurotransmitter- and hormone-containing vesicles with the plasma membrane. They cleave the three SNARE proteins: **VAMP**, SNAP-25 and syntaxin, and this selective proteolysis results in a blockade of exocytosis. The Helicobacter pylori cytotoxin is implicated in the pathogenesis of gastroduodenal ulcers. It causes a progressive and extensive vacuolation of cells

followed by necrosis, after a cytotoxin-induced alteration of membrane trafficking by late endosomes. Vacuoles originate from this compartment in a rab7-dependent process and swell because they are acidic and accumulate membrane-permeant amines.

L24 ANSWER 17 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1998:133411 CAPLUS
TI Measurement of a **synaptobrevin**-thioredoxin fusion protein (VAMPII(51aa)-T) by capillary zone electrophoresis using laser induced fluorescence detection
AU Asermely, Karen E.; Nowakowski, Janet; Courtney, Bernard C.; Adler, Michael
CS Neurotoxicology Branch, Pathophysiology Div., U.S. Army Med. Research Inst. Chem. Defense, Aberdeen Proving Ground, MD, 21010-5425, USA
SO Med. Def. Biosci. Rev., Proc. (1996), Volume 2, 751-756 Publisher: National Technical Information Service, Springfield, Va. CODEN: 64UTAN
DT Conference
LA English
AB **Botulinum Toxin** B (BoNT/B) has endopeptidase activity and cleaves **synaptobrevin** II, Vesicle Assocd. Membrane Protein II (**VAMP** II) in neurons. The long-term goal of these studies is to find a drug which will inhibit the endopeptidase activity of BoNT/B at the Q(Gln 76) -F(Phe 77) site in VAMPII. VAMPII is a protein contg. 116 aa. Smaller fragments of VAMPII have been used to study the BoNT/B effect of cleavage. In this study a 51 aa fragment of VAMPII was cloned in E. coli and expressed as a **synaptobrevin**-thioredoxin fusion protein, VAMPII (51aa)-T. Capillary Zone Electrophoresis (CZE) was used to identify VAMPII (51aa)-T. The migration time of VAMPII(51aa)-T was detd. under various conditions of pH and applied voltage. The optimal migration time obtained was 7.8 min at pH 9.0 and applied voltage of 30 kV. This is the first paper to describe the measurement of VAMPII (51aa)-T by CZE. In future studies this CZE method will be used to monitor the cleavage of VAMPII fragments by BoNT/B and identify potential inhibitory drugs.

L24 ANSWER 18 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 96202254 EMBASE
TI Cleavage of vesicle-associated membrane protein (**VAMP**)-2 and cellubrevin on GLUT4-containing vesicles inhibits the translocation of GLUT4 in 3T3-L1 adipocytes.
AU Tamori Y.; Hashiramoto M.; Araki S.; Kamata Y.; Takahashi M.; Kozaki S.; Kasuga M.
CS Centre Molecular Cellular Biology, University of Queensland, Brisbane, QLD 4072, Australia
SO Biochemical and Biophysical Research Communications, (1996) 220/3 (740-745).
ISSN: 0006-291X CODEN: BBRCA
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB We have identified **VAMP** isoforms, **VAMP**-2 and cellubrevin, on GLUT4-containing vesicle membranes isolated from 3T3-L1 adipocytes. These proteins translocate from a low density microsomal fraction to the plasma membrane upon insulin stimulation in a fashion similar to GLUT4. **VAMP**-1 was not detected in this low density microsomal fraction nor on purified GLUT4-containing vesicles. In streptolysin-O permeabilized 3T3-L1 adipocytes, both **VAMP**-2 and cellubrevin were cleaved with **botulinum** neurotoxin isoform B, BoNTx/B. In addition, BoNTx/B partially inhibited insulin-stimulated GLUT4 translocation

and glucose transport activity. We conclude that the **synaptobrevin** isoforms are important components of the insulin-dependent translocation of GLUT4 to the cell surface in adipocytes.

L24 ANSWER 19 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1996:655092 CAPLUS
DN 125:295025
TI Cleavage of SNARE proteins is correlated to inhibition of neuroexocytosis by tetanus and **botulinum** neurotoxins and SNARE proteins are present in non neuronal tissues
AU Rossetto, O.; Osen-Sand, A.; Catsicas, S.; Naldi, E.; Malgaroli, A.; Schiavo, G.; Montecucco, C.
CS Department Biomedical Sciences, University Padova, Padua, 35121, Italy
SO Zentralbl. Bakteriol., Suppl. (1996), 28(Bacterial Protein Toxins), 260-268
CODEN: ZBASE2; ISSN: 0941-018X
DT Journal
LA English
AB Clostridial neurotoxins were recently shown to be zinc-endopeptidases specific for three proteins of the neuroexocytosis app. Tetanus neurotoxin (TeNT) and **botulinum** neurotoxins (BoNT) type B, D, F and G recognize and cleave at single peptide bonds **VAMP**/**synaptobrevin**, an integral membrane protein of neurotransmitter contg. small synaptic vesicles whereas BoNT/A, /C and /E are specific for proteins of the presynaptic membrane. BoNT/A and /E cleave two different peptide bonds at the carboxy-terminal of SNAP-25, while BoNT/C cleaves syntaxin. We found that BoNT/C is also able to cleave SNAP-25. Here, we correlate inhibition of neurotransmitter release to proteolysis of the three **toxin** targets in cortical rat neurons intoxicated with BoNT/A, /B and /C. Our results indicate that **VAMP**, SNAP-25 and syntaxin play a key role in neuroexocytosis. We also demonstrate that **VAMP**/**synaptobrevin**, the target of five out of eight clostridial neurotoxins, is widely and differentially expressed in non neuronal tissue.

L24 ANSWER 20 OF 54 MEDLINE
AN 97014184 MEDLINE
DN 97014184
TI Tetanus and botulism neurotoxins: a novel group of zinc-endopeptidases.
AU Tonello F; Morante S; Rossetto O; Schiavo G; Montecucco C
CS Centro CNR Biomembrane and Dipartimento di Scienze Biomediche, Universita di Padov`a, Italy.
SO ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1996) 389 251-60.
Ref: 47
Journal code: 2LU. ISSN: 0065-2598.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199705
EW 19970502
AB Tetanus and **botulinum** neurotoxins are produced by bacteria of the genus Clostridium and cause the paralytic syndromes of tetanus and botulism with a persistent inhibition of neurotransmitter release at central and peripheral synapses, respectively. These neurotoxins consist of two disulfide-linked polypeptides: H (100 kDa) is responsible for neurospecific binding

and cell penetration of L(50 kDa), a zinc-endopeptidase specific for three protein subunits of the neuroexocytosis apparatus. Tetanus neurotoxin and **botulinum** neurotoxins serotypes B, D, F, and G cleave at single sites, which differ for each neurotoxin. **VAMP/synaptobrevin**, a membrane protein of the synaptic vesicles. **Botulinum** A and E neurotoxins cleave SNAP-25, a protein of the presynaptic membrane, at two different carboxyl-terminal peptide bonds. Serotype C cleaves specifically syntaxin, another protein of the nerve plasmalemma. The target specificity of these metallo-proteinases relies on a double recognition of their substrates based on interactions with the cleavage site and with a non contiguous segment that contains a structural motif common to **VAMP**, SNAP-25 and syntaxin.

L24 ANSWER 21 OF 54 MEDLINE
AN 96271580 MEDLINE
DN 96271580
TI Common and distinct fusion proteins in axonal growth and transmitter release.
AU Osen-Sand A; Staple J K; Naldi E; Schiavo G; Rossetto O; Petitpierre S; Malgaroli A; Montecucco C; Catsicas S
CS Glaxo Institute for Molecular Biology, Geneva, Switzerland.
SO JOURNAL OF COMPARATIVE NEUROLOGY, (1996 Apr 1) 367 (2) 222-34. Journal code: HUV. ISSN: 0021-9967.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611
AB We have used the proteolytic properties of **botulinum** and tetanus neurotoxins (BoNT, TeNT) to cleave three proteins of the membrane fusion machinery, SNAP-25, **VAMP/synaptobrevin**, and syntaxin, in developing and differentiated rat central neurons in vitro. Then, we have studied the capacity of neurons to extend neurites, make synapses, and release neurotransmitters. All the toxins showed the expected specificity with the exception that BoNT/C cleaved SNAP-25 in addition to syntaxin and induced rapid neuronal death. In developing neurons, cleavage of SNAP-25 with BoNT/A inhibited axonal growth and prevented synapse formation. In contrast, cleavage of **VAMP** with TeNT or BoNT/B had no effects on neurite extension and synaptogenesis. All the toxins tested inhibited transmitter release in differentiated neurons, and cleavage of **VAMP** resulted in the strongest inhibition. These data indicate that SNAP-25 is involved in vesicle fusion for membrane expansion and transmitter release, whereas **VAMP** is selectively involved in transmitter release. In addition, our results support the hypothesis that synaptic activity is not essential for synapse formation in vitro.

L24 ANSWER 22 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1997:49810 CAPLUS
DN 126:71293
TI Tetanus and **botulinum** neurotoxins
AU Schiavo, Giampietro; Rossetto, Ornella; Montecucco, Cesare
CS Dipto. die Scienze Biomediche, Univ. di Padova, Padua, 35121, Italy
SO Zinc Metalloproteases Health Dis. (1996), 205-220. Editor(s): Hooper, Nigel M. Publisher: Taylor & Francis, London, UK. CODEN: 63WOAB
DT Conference; General Review
LA English
AB A review and discussion with many refs. Tetanus and **botulinum** neurotoxins are produced by anaerobic bacteria of the genus Clostridium and cause the paralytic syndromes of tetanus and botulism. They are synthesized as a single inactive 150-kDa

polypeptide chain and activated by specific proteolysis with the generation of 2 disulfide-linked polypeptides, termed H and L. The larger chain H is responsible for neuro-specific binding and cell penetration. Redn. releases the L chain in the neuronal cytosol, where it displays its catalytic activity. The L chain is a zinc endopeptidase specific for protein components of the neuro-exocytosis app. Tetanus neurotoxin and **botulinum** neurotoxin serotypes B, D, F, and G recognize specifically **VAMP/synaptobrevin**, an integral membrane protein of the synaptic vesicles. **Botulinum** A and E neurotoxins recognize and cleave specifically SNAP-25, a protein of the presynaptic membrane, whereas serotype C cleaves specifically syntaxin, another protein of the nerve plasmalemma. These 3 protein targets are cleaved at single sites, which differ for each neurotoxin. The fact that neurotoxins that cause a persistent blockade of neuro-exocytosis attack **VAMP**, SNAP-25, and syntaxin indicates that these 3 proteins play an important role in the process. The unique sequence, mechanism of activation, and target specificity of tetanus and **botulinum** neurotoxins individualize them as an independent group of zinc endopeptidases.

L24 ANSWER 23 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 96067634 EMBASE
TI [Molecular mechanims of tetanus and **botulinum** neurotoxins].
MODES D'ACTION MOLCULAIRE DES NEUROTOXINES BOTULIQUES ET TETANIQUE.
AU Deloye F.; Schiavo G.; Doussau F.; Rossetto O.; Montecucco C.; Poulain B.
CS Laboratoire neurobiologie cellulaire, UPR-Cnrs 9009, centre de neurochimie, 5 rue Blaise-Pascal, 67084 Strasbourg Cedex, France
SO Medecine/Sciences, (1996) 12/2 (175-182).
ISSN: 0767-0974 CODEN: MSMSE4
CY France
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
052 Toxicology
LA French
SL French; English
AB Tetanus (TeNT) and **botulinum** (BoNTs, seven serotypes A-G) neurotoxins are the causal agents of two severe diseases, tetanos and botulism. The TeNT blocks preferentially GABA or glycine release in the central nervous system whereas, BoNTs inhibit acetylcholine release in periphery. These neurotoxins are proteins constituted of a heavy and a light chains. The heavy chain mediates specific binding of toxins to neurone and translocation of light chain into the cytoplasm. The light chain alone is responsible for the intraneuronal blockade of neurotransmitter release. Recently, the light chain was found to be a zinc-endopeptidase. It attacks specifically synaptic proteins of the neuro-exocytotic apparatus. TeNT and BoNT/B, D, F and /G cleave **VAMP**/**synaptobrevin** an integral protein of the synaptic BoNT/E attack specifically SNAP-25, a protein associated to the plasma membrane. BoNT/G cleaves HPC1/syntaxin, an integral protein of the plasma membrane that is associated to the calcium channels implicated to the calcium channels implicated in neurotransmitter release.

L24 ANSWER 24 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 11
AN 96166046 EMBASE
TI Substrate residues N-terminal to the cleavage site of **botulinum** type B neurotoxin play a role in determining the specificity of its endopeptidase activity.
AU Wictome M.; Rossetto O.; Montecucco C.; Shone C.C.

CS Centre for Applied Microbiology/Res., Porton Down, Sallisbury, Wiltshire SP4 0JG, United Kingdom
SO FEBS Letters, (1996) 386/2-3 (133-136).
ISSN: 0014-5793 CODEN: FEBLAL
CY Netherlands
DT Journal
FS 004 Microbiology
008 Neurology and Neurosurgery
052 Toxicology
LA English
SL English
AB Clostridium **botulinum** type B neurotoxin is a highly specific zinc-endopeptidase which cleaves vesicle-associated membrane protein (**VAMP/synaptobrevin**), a critical component of the vesicle docking/fusion mechanism. In this study, substrate residues flanking the N-terminal side of the cleavage site are shown to play a key role in enzyme substrate recognition. Two aspartate residues in this region are identified as critical determinants of the neurotoxin's specificity. These findings are discussed in relation to the mechanism by which **botulinum** type B neurotoxin cleaves its substrate.

L24 ANSWER 25 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95186965 EMBASE
TI Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including **VAMP**, NSF, SNAP, annexins, and GTPases.
AU Schnitzer J.E.; Liu J.; Oh P.
CS Research North-Beth Israel, Dept. of Pathology, Harvard Medical School, 99 Brookline Ave., Boston, MA 02215, United States
SO Journal of Biological Chemistry, (1995) 270/24 (14399-14404).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Transport by discrete vesicular carriers is well established at least in part because of recent discoveries identifying key protein mediators of vesicle formation, docking, and fusion. A general mechanism sensitive to N- ethylmaleimide (NEM) is required for the transport of a divergent group of vesicular carriers in all eukaryotes. Many endothelia have an abundant population of noncoated plasmalemmal vesicles or caveolae, which have been reported with considerable controversy to function in transport. We recently have shown that like other vesicular transport systems, caveolae-mediated endocytosis and transcytosis are inhibited by NEM (Schnitzer, J. E., Allard, J., and Oh, P. (1995) Am. J. Physiol. 268, H48-H55). Here, we continue this work by utilizing our recently developed method for purifying endothelial caveolae from rat lung tissue (Schnitzer, J. E., Oh, P., Jacobson, B. S., and Dvorak, A. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1759-1763) to show that these caveolae contain key proteins known to mediate different aspects of vesicle formation, docking, and/or fusion including the vSNARE **VAMP** -2, monomeric and trimeric GTPases, annexins II and VI, and the NEM-sensitive fusion factor NSF along with its attachment protein SNAP. Like neuronal VAMPs, this endothelial **VAMP** is sensitive to cleavage by **botulinum** B and tetanus neurotoxins. Caveolae in endothelium are indeed like other carrier vesicles and contain similar NEM-sensitive molecular machinery for transport.

L24 ANSWER 26 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 12
AN 95164413 EMBASE

TI Disassembly of the reconstituted synaptic vesicle membrane fusion complex in vitro.
AU Hayashi T.; Yamasaki S.; Nauenburg S.; Binz T.; Niemann H.
CS Department of Microbiology, Fed Res Ctr Virus Diseases Animals, Paul Ehrlich Strasse 28, D72076 Tubingen, Germany, Federal Republic of
SO EMBO Journal, (1995) 14/10 (2317-2325).
ISSN: 0261-4189 CODEN: EMJODG
CY United Kingdom
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB The interaction of the presynaptic membrane proteins SNAP-25 and syntaxin with the synaptic vesicle protein **synaptobrevin** (**VAMP**) plays a key role in the regulated exocytosis of neurotransmitters. Clostridial neurotoxins, which proteolyze these polypeptides, are potent inhibitors of neurotransmission. The cytoplasmic domains of the three membrane proteins join into a tight SDS-resistant complex. Here, we show that this reconstituted complex, as well as heterodimers composed of syntaxin and SNAP-25, can be disassembled by the concerted action of the N-ethylmaleimide-sensitive factor, NSF, and the soluble NSF attachment protein, .alpha.-SNAP .alpha.-SNAP binds to predicted .alpha.-helical coiled-coil regions of syntaxin and SNAP-25, shown previously to be engaged in their direct interaction. **Synaptobrevin**, although incapable of binding .alpha.-SNAP individually, induced a third .alpha.-SNAP binding site when associated with syntaxin and SNAP-25 into heterotrimers. NSF released prebound .alpha.-SNAP from full-length syntaxin but not from a syntaxin derivative truncated at the N-terminus. Disassembly of complexes containing this syntaxin mutant was impaired, indicating a critical role for the N-terminal domain in the .alpha.-SNAP/NSF-mediated dissociation process. Complexes containing C-terminally deleted SNAP-25 derivatives, as generated by **botulinal** toxins type A and E, were dissociated more efficiently. In contrast, the N-terminal fragment generated from **synaptobrevin** by **botulinal toxin** type F produced an SDS-sensitive complex that was poorly dissociated.

L24 ANSWER 27 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95285799 EMBASE
TI Phosphorylation of **VAMP/synaptobrevin** in synaptic vesicles by endogenous protein kinases.
AU Nielander H.B.; Onofri F.; Valtorta F.; Schiavo G.; Montecucco C.; Greengard P.; Benfenati F.
CS Section of Physiology, Department of Biomedical Sciences, University of Modena, Via Campi 287, I-41100 Modena, Italy
SO Journal of Neurochemistry, (1995) 65/4 (1712-1720).
ISSN: 0022-3042 CODEN: JONRA
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
LA English
SL English
AB **VAMP/synaptobrevin** (SYB), an integral membrane protein of small synaptic vesicles, is specifically cleaved by tetanus neurotoxin and **botulinum** neurotoxins B, D, F, and G and is thought to play an important role in the docking and/or fusion of synaptic vesicles with the presynaptic membrane. Potential phosphorylation sites for various kinases are present in SYB sequence. We have studied whether SYB is a substrate for protein kinases that are present in nerve terminals and known to modulate neurotransmitter release. SYB can be phosphorylated within the same vesicle by endogenous Ca2+/calmodulin-dependent protein kinase II

(CaMKII) associated with synaptic vesicles. This phosphorylation reaction occurs rapidly and involves serine and threonine residues in the cytoplasmic region of SYB. Similarly to CaMKII, a casein kinase II (CasKII) activity copurifying with synaptic vesicles is able to phosphorylate SYB selectively on serine residues of the cytoplasmic region. This phosphorylation reaction is markedly stimulated by sphingosine, a sphingolipid known to activate CasKII and to inhibit CaMKII and protein kinase C. The results show that SYB is a potential substrate for protein kinases involved in the regulation of neurotransmitter release and open the possibility that phosphorylation of SYB plays a role in modulating the molecular interactions between synaptic vesicles and the presynaptic membrane.

L24 ANSWER 28 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 13
AN 96010686 EMBASE
TI Inhibition by clostridial neurotoxins of calcium independent [3H]noradrenaline outflow from freeze thawed synaptosomes: Comparison with **synaptobrevin** hydrolysis.
AU Hausinger A.; Volknandt W.; Zimmermann H.; Habermann E.
CS Germany, Federal Republic of
SO Toxicon, (1995) 33/11 (1519-1530).
ISSN: 0041-0101 CODEN: TOXIA6
CY United Kingdom
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB Clostridial neurotoxins are known to inhibit regulated, i.e. calcium dependent exocytosis. In the present study we have investigated their potential role in also inhibiting calcium independent exocytosis. Synaptosomes from rat forebrain were preloaded with [3H]noradrenaline and permabilized reversibly by freezing in Ca2+ free potassium glutamate containing dimethyl sulfoxide and the toxins to be assayed. Subsequently, outflow of radioactivity was measured in isotonic calcium free potassium glutamate. The synaptic vesicle protein **synaptobrevin** 2/**VAMP** 2 and its **toxin** dependent degradation were analysed by Western blotting. The light chain of tetanus **toxin** reduced the synaptosomal outflow of radioactivity, whereas the activity of heavy chain was at the detection limit. The respective activities of the dichain toxins from Clostridium tetani and C. **botulinum** A, B and E were enhanced by pretreatment with dithiothreitol. Reduced single chain tetanus **toxin** was less potent than reduced dichain tetanus **toxin**. Pretreatment with ethylene diamine tetraacetic acid as an inhibitor of Zn2+ proteases abolished the actions of the tetanus toxic light chain and of the reduced dichain toxins. Hydrolysis of **synaptobrevin** 2/**VAMP** 2 was obtained with tetanus **toxin** light chain, reduced dichain tetanus **toxin** and C. **botulinum** B **toxin** . Its hydrolysis by single chain tetanus **toxin** was less pronounced, and it was absent with **botulinum** toxins A and E. It is concluded that clostridial neurotoxins can not only inhibit calcium-dependent release but also affect calcium-independent outflow from synaptosomes. Since this is accompanied by selective intrasynaptosomal proteolysis of **synaptobrevin**, calcium-independent outflow may at least in part involve the vesicular release apparatus.

L24 ANSWER 29 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95028932 EMBASE
TI Vesicle-associated membrane protein (**VAMP**)/ **synaptobrevin**-2 is associated with dense core secretory

granules in PC12 neuroendocrine cells.
AU Papini E.; Rossetto O.; Cutler D.F.
CS MRC Lab. for Molecular Cell Biology, University College London, Gower St., WC1E 6BT London, United Kingdom
SO Journal of Biological Chemistry, (1995) 270/3 (1332-1336). ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB The presence and intracellular distribution of vesicle-associated membrane protein-1 (**VAMP**-1) and **VAMP**-2 were investigated in the PC12 neuroendocrine cell line using isotype-specific polyclonal antibodies. **VAMP**-2 was detected in the total membrane fraction, while **VAMP**-1 was undetectable. Subcellular fractionation demonstrates that a substantial amount of the **VAMP**-2 (24-36%) is associated with dense core, catecholamine-containing granules (DCGs). This was confirmed by immunofluorescence microscopy. The L chain of tetanus neurotoxin, known to inhibit granule mediated secretion in permeabilized PC12 cells, as well as **botulinum** neurotoxins F and G, effectively cleaved DCG- associated **VAMP**-2. These data demonstrate that **VAMP**-2 is present on the secretory granules of PC12 cells.

L24 ANSWER 30 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95052082 EMBASE
TI Vesicle-associated membrane protein-2 (**synaptobrevin**-2) forms a complex with synaptophysin.
AU Washbourne P.; Schiavo G.; Montecucco C.
CS Centro CNR Biomembrane, Dipartimento di Scienze Biomediche, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO Biochemical Journal, (1995) 305/3 (721-724). ISSN: 0264-6021 CODEN: BIJOAK
CY United Kingdom
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB Vesicle-associated membrane protein (**VAMP**) (or **synaptobrevin**), a type II membrane protein of small synaptic vesicles, is essential for neuroexocytosis because its proteolysis by tetanus and **botulinum** neurotoxins types B, D, F and G blocks neurotransmitter release. The addition of cross-linking reagents to isolated small synaptic vesicles induces the formation of 30 and 50 kDa complexes containing the isoform 2 of **VAMP** (**VAMP**-2). Whereas the 30 kDa band is a **VAMP**-2 homodimer, the 50 kDa species results from the cross-linking of **VAMP**-2 with synaptophysin. This heterodimer also forms in detergent-solubilized vesicles and involves the N-terminal part of **VAMP**-2. The implications of the existence of a synaptophysin-**VAMP**-2 complex in the processes of vesicle docking and fusion with the presynaptic membrane are discussed.

L24 ANSWER 31 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 14
AN 95094898 EMBASE
TI Calcium-dependent endogenous proteolysis of the vesicle proteins **synaptobrevin** and synaptotagmin.
AU Hausinger A.; Volknandt W.; Zimmermann H.
CS AK Neurochemie, Zoologisches Institut, Biozentrum J.W. Goethe-Universitat, Marie-Curie-Strasse 9, D-60439 Frankfurt am Main, Germany, Federal Republic of

SO NeuroReport, (1995) 6/4 (637-641).
ISSN: 0959-4965 CODEN: NERPEZ
CY United Kingdom
DT Journal
FS 002 Physiology
037 Drug Literature Index
LA English
SL English
AB The synaptic vesicle integral protein **synaptobrevin/VAMP** is a target of the clostridial metalloproteases tetanus **toxin** and **botulinum** toxins. We provide evidence that **synaptobrevin** can also be cleaved by an endogenous protease. As revealed by Western blotting proteolysis is calcium-dependent, results in the formation of an 8 kD peptide that becomes apparent within 10 min. Proteolysis can be inhibited by the chelating agents EGTA and EDTA, whereas other protease inhibitors failed to prevent degradation. In addition, a proteolytic degradation of the synaptic vesicle specific protein synaptotagmin could be observed. Other proteins including the synaptic vesicle proteins synapsin I and synaptophysin remained unaltered. Partial calcium-dependent degradation of select synaptic vesicle proteins may play a role in the life cycle of the organelle.

L24 ANSWER 32 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 15
AN 95327327 EMBASE
TI Bacterial neurotoxins - A thousand years later.
AU Linial M.
CS Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, 91904 Jerusalem, Israel
SO Israel Journal of Medical Sciences, (1995) 31/10 (591-595).
ISSN: 0021-2180 CODEN: IJMDAI
CY Israel
DT Journal
FS 004 Microbiology
LA English
SL English
AB Clostridium bacteria are responsible for the neuroparalysis in tetanus and in botulism by producing potent neurotoxins. Here we review the current developments in understanding the toxins' mode of action by deciphering the molecular basis for their function. The active forms of tetanus and **botulinum** neurotoxins block neurotransmitter release via a zinc-dependent protease activity. All known tetanus and **botulinum** toxins cleave only three key components in the synaptic vesicle docking and fusion protein complex. While tetanus and **botulinum** types B, D, F and G cleave **VAMP/synaptobrevin**, an integral membrane protein of the synaptic vesicles, two other synaptic proteins from the plasma membrane, SNAP-25 and syntaxin, are cleaved by **botulinum** types A and E and **botulinum** type C, respectively. We discuss the mechanism by which the proteolytic activity of these toxins causes a block in vesicle fusion.

L24 ANSWER 33 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 16
AN 96022076 EMBASE
TI Structure and function of tetanus and **botulinum** neurotoxins.
AU Montecucco C.; Schiavo G.
CS Centro CNR Biomembrane, Dipartimento di Scienze Biomediche, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO Quarterly Reviews of Biophysics, (1995) 28/4 (423-472).
ISSN: 0033-5835 CODEN: QURBAW
CY United Kingdom
DT Journal

FS 004 Microbiology
008 Neurology and Neurosurgery
029 Clinical Biochemistry
052 Toxicology
037 Drug Literature Index
LA English
SL English
AB Tetanus and **botulinum** neurotoxins are produced by Clostridia and cause the neuroparalytic syndromes of tetanus and botulism. Tetanus neurotoxin acts mainly at the CNS synapse, while the seven **botulinum** neurotoxins act peripherally. Clostridial neurotoxins share a similar mechanism of cell intoxication: they block the release of neurotransmitters. They are composed of two disulfide-linked polypeptide chains. The larger subunit is responsible for neurospecific binding and cell penetration. Reduction releases the smaller chain in the neuronal cytosol, where it displays its zinc-endopeptidase activity specific for protein components of the neuroexocytosis apparatus. Tetanus neurotoxin and **botulinum** neurotoxins B, D, F and G recognize specifically **VAMP/synaptobrevin**. This integral protein of the synaptic vesicle membrane is cleaved at single peptide bonds, which differ for each neurotoxin. **Botulinum** A, and E neurotoxins recognize and cleave specifically SNAP-25, a protein of the presynaptic membrane, at two different sites within the carboxyl-terminus. **Botulinum** neurotoxin type C cleaves syntaxin, another protein of the nerve plasmalemma. These results indicate that **VAMP**, SNAP-25 and syntaxin play a central role in neuroexocytosis. These three proteins are conserved from yeast to humans and are essential in a variety of docking and fusion events in every cell. Tetanus and **botulinum** neurotoxins form a new group of zinc-endopeptidases with characteristic sequence, mode of zinc coordination, mechanism of activation and target recognition. They will be of great value in the unravelling of the mechanisms of exocytosis and endocytosis, as they are in the clinical treatment of dystonias.

L24 ANSWER 34 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1995:964529 CAPLUS
DN 124:2582
TI Intracellular targets and metalloprotease activity of tetanus and botulism neurotoxins
AU Schiavo, G.; Rossetto, O.; Tonello, F.; Montecucco, C.
CS Centro CNR Biomembrane, Universita di Padova, Padva, 35121, Italy
SO Curr. Top. Microbiol. Immunol. (1995), Volume Date 1995, 195, 257-74
CODEN: CTMIA3; ISSN: 0070-217X
DT Journal; General Review
LA English
AB A review and discussion with several refs. on structural aspects of Clostridial neurotoxins, structure of the L chain, metalloproteinase activity, **VAMP/synaptobrevin**, SNAP-25, syntaxin, neuroexocytosis app. and Clostridial neurotoxins, and future perspectives.

L24 ANSWER 35 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95239699 EMBASE
TI Role of myosin in neurotransmitter release: Functional studies at synapses formed in culture.
AU Mochida S.
CS Department of Phsyiology, Tokyo Medical College, 1-1, Shinjuku-6-chome, Shinjuku-ku, Tokyo 160, Japan
SO Journal of Physiology Paris, (1995) 89/2 (83-94).
ISSN: 0928-4257 CODEN: JHYSEM
CY France
DT Journal

FS 002 Physiology
037 Drug Literature Index
LA English
SL English
AB To determine the functional role of presynaptic proteins in the neurotransmitter release, I have employed cholinergic synapses formed between superior cervical ganglion neurons in culture. These synapses expressed proteins characteristic of mature synapses: immunofluorescence staining showed the presence of synaptophysin, synaptotagmin, **VAMP/synaptobrevin**-2, syntaxin and neurexin. The function of these proteins seems to be similar to that of mature synapses because **botulinum** neurotoxins A, E and C1 inhibited neurotransmitter release evoked by presynaptic action potentials. With this preparation, I have obtained evidence supporting roles for myosin II and myosin light chain kinase in neurotransmitter secretion. Acetylcholine release was inhibited by introduction of antibody against myosin II or inhibitors of myosin light chain kinase. This evidence suggests a model in which myosin light chain kinase phosphorylates myosin, and the resultant change in actin-myosin interactions is involved in some steps of neurotransmitter release.

L24 ANSWER 36 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 17
AN 95239694 EMBASE
TI The metallo-proteinase activity of tetanus and botulism neurotoxins.
AU Rossetto O.; Deloye F.; Poulain B.; Pellizzari R.; Schiavo G.; Montecucco C.
CS Centro CNR Biomembrane, Dipartimento di Scienze Biomediche, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO Journal of Physiology Paris, (1995) 89/1 (43-50).
ISSN: 0928-4257 CODEN: JHYSEM
CY France
DT Journal
FS 002 Physiology
008 Neurology and Neurosurgery
029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB Tetanus and **botulinum** neurotoxins are produced by several Clostridia and cause the paralytic syndromes of tetanus and botulism by blocking neurotransmitter release at central and peripheral synapses, respectively. They consist of two disulfide-linked polypeptides: H (100 kDa) is responsible for neurospecific binding and cell penetration of L (50 kDa), a zinc-endopeptidase specific for three protein subunits of the neuroexocytosis apparatus. Tetanus neurotoxin and **botulinum** neurotoxin serotypes B, D, F and G cleave at single sites, which differ for each neurotoxin, **VAMP/synaptobrevin**, a membrane protein of the synaptic vesicles. **Botulinum** A and E neurotoxins cleave SNAP-25, a protein of the presynaptic membrane, at two different carboxyl-terminal peptide bonds. Serotype C cleaves specifically syntaxin, another protein of the nerve plasmalemma. The target specificity of these metallo-proteinases relies on a double recognition of their substrates based on interactions with the cleavage site and with a non-contiguous segment that contains a structural motif common to **VAMP**, SNAP-25 and syntaxin.

L24 ANSWER 37 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 18
AN 94257448 EMBASE
TI **Botulinum** G neurotoxin cleaves **VAMP/synaptobrevin** at a single Ala-Ala peptide bond.
AU Schiavo G.; Malizio C.; Trimble W.S.; De Laureto P.P.; Milan G.;

Sugiyama H.; Johnson E.A.; Montecucco C.
CS Dipartimento di Scienze Biomediche, CCNRB, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO J. BIOL. CHEM., (1994) 269/32 (20213-20216).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Similarly to other serotypes, **botulinum** neurotoxin serotype G (BoNT/G) contains the zinc binding motif of zinc endopeptidases. Highly purified preparations of BoNT/G show a zinc-dependent protease activity specific for **VAMP/synaptobrevin**, a membrane protein of synaptic vesicles. The two neuronal **VAMP** isoforms are cleaved with similar rates at one Ala-Ala peptide bond present in the same region, out of the several such peptide bonds present in their sequences. This site of cleavage is unique among the eight clostridial neurotoxins. **VAMP** proteolysis is displayed only after reduction of the single interchain disulfide bond present in the **toxin**, and it is inhibited by EDTA, o-phenanthroline and captopril.

L24 ANSWER 38 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1994:527516 CAPLUS
DN 121:127516
TI **Botulinum** G neurotoxin cleaves **VAMP/synaptobrevin** at a single Ala-Ala peptide bond
AU Schiavo, Giampietro; Malizio, Carl; Trimble, William S.; Polverino de Laureto, Patrizia; Milan, Gabriella; Sugiyama, Hiroshi; Johnson, Eric A.; Montecucco, Cesare
CS Centro Consiglio Nazionale delle Richerche Biomembrane, Padua, 35121, Italy
SO J. Biol. Chem. (1994), 239(32), 20213-16
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB Similarly to other serotypes, **botulinum** neurotoxin serotype G (BoNT/G) contains the zinc binding motif of zinc endopeptidases. Highly purified prepns. of BoNT/G show a zinc-dependent protease activity specific for **VAMP/synaptobrevin**, a membrane protein of synaptic vesicles. The two neuronal **VAMP** isoforms are cleaved with similar rates at one Ala-Ala peptide bond present in the same region, out of the several such peptide bonds present in their sequences. This site of cleavage is unique among the eight clostridial neurotoxins. **VAMP** proteolysis is displayed only after redn. of the single interchain disulfide bond present in the **toxin**, and it is inhibited by EDTA, o-phenanthroline and captopril.

L24 ANSWER 39 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 19
AN 94206328 EMBASE
TI Cleavage of members of the **synaptobrevin/VAMP** family by types D and F **botulinal** neurotoxins and tetanus **toxin**.
AU Yamasaki S.; Baumeister A.; Binz T.; Blasi J.; Link E.; Cornille F.; Roques B.; Fykse E.M.; Sudhof T.C.; Jahn R.; Niemann H.
CS Department of Microbiology, Federal Virus Animals Dis. Res. Ctr., P. O. Box 1149, D-72001 Tubingen, Germany, Federal Republic of
SO J. BIOL. CHEM., (1994) 269/17 (12764-12772).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 004 Microbiology

LA English
SL English
AB Tetanus **toxin** (TeTx) and the various forms of **botulinal** neurotoxins (BoNT/A to BoNT/G) potently inhibit neurotransmission by means of their L chains which selectively proteolyze synaptic proteins such as **synaptobrevin** (TeTx, BoNT/B, BoNT/F), SNAP-25 (BoNT/A), and syntaxin (BoNT/C1). Here we show that BoNT/D cleaves rat **synaptobrevin** 1 and 2 in toxified synaptosomes and in isolated vesicles. In contrast, **synaptobrevin** 1, as generated by in vitro translation, is only a poor substrate for BoNT/D, whereas this species is cleaved by BoNT/F with similar potency. Cleavage by BoNT/D occurs at the peptide bond Lys59-Leu60 which is adjacent to the BoNT/F cleavage site (Gln58-Lys59) and again differs from the site hydrolyzed by TeTx and BoNT/B (Gln76-Phe77). Cellubrevin, a recently discovered isoform expressed outside the nervous system, is efficiently cleaved by all three toxins examined. For further characterization of the substrate requirements of BoNT/D, we tested amino- and carboxyl-terminal deletion mutants of **synaptobrevin** 2 as well as synthetic peptides. Shorter peptides containing up to 15 amino acids on either side of the cleavage site were not cleaved, and a peptide extending from Arg47 to Thr116 was a poor substrate for all three toxins tested. However, cleavability was restored when the peptide is further extended at the NH2 terminus (Thr27-Thr116) demonstrating that NH2 terminally located sequences of **synaptobrevin** which are distal from the respective cleavage sites are required for proteolysis. To further examine the isoform specificity, several mutants of rat **synaptobrevin** 2 were generated in which individual amino acids were replaced with those found in rat **synaptobrevin** 1. We show that a Met46 to Ile46 substitution drastically diminishes cleavability by BoNT/D and that the presence of Val76 instead of Gln76 dictates the reduced cleavability of **synaptobrevin** isoforms by TeTx.

L24 ANSWER 40 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 20
AN 94167193 EMBASE
TI **Synaptobrevin**/vesicle-associated membrane protein (**VAMP**) of Aplysia californica: Structure and proteolysis by tetanus **toxin** and **botulinal** neurotoxins type D and F.
AU Yamasaki S.; Hu Y.; Binz T.; Kalkuhl A.; Kurazono H.; Tamura T.; Jahn R.; Kandel E.; Niemann H.
CS FRCVDA, Paul-Ehrlich-Strasse 28, D-72076 Tubingen, Germany, Federal Republic of
SO PROC. NATL. ACAD. SCI. U. S. A., (1994) 91/11 (4688-4692). ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal
FS 004 Microbiology
LA English
SL English
AB **Synaptobrevin**/vesicle-associated membrane protein (**VAMP**) and syntaxin are potential vesicle donor and target membrane receptors of a docking complex that requires N-ethylmaleimide-sensitive factor (NSF) and soluble NSF- attachment proteins as soluble factors for vesicle fusion with target membranes. Members of this docking complex are the target of clostridial neurotoxins that act as zinc-dependent proteases. Molecular cloning of the Aplysia californica **synaptobrevin** cDNA revealed a 180-residue polypeptide (M(r), 19,745) with a central transmembrane region and an atypically large C- terminal intravesicular domain. This polypeptide integrates into membranes at both the co- and posttranslational level, as shown by modification of an artificially introduced N-glycosylation site. The soluble and

membrane- anchored forms of **synaptobrevin** are cleaved by the light chains of the **botulinal** toxins type D and F and by tetanus **toxin** involving the peptide bonds Lys49-Ile50, Gln48-Lys49, and Gln66-Phe67, respectively. The active center of the tetanus **toxin** light chain was identified by site- specific mutagenesis. His233, His237, Glu234, and Glu(270/271) are essential to this proteolytic activity. Modification of histidine residues resulted in loss of zinc binding, whereas a replacement of Glu234 only slightly reduced the zinc content.

L24 ANSWER 41 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1994:317569 CAPLUS
DN 120:317569
TI **Botulinum** neurotoxin type G proteolyses the Ala81-Ala82 bond of rat **synaptobrevin** 2
AU Yamasaki, Shinji; Binz, Thomas; Hayashi, Tetsuya; Szabo, Elizabeth; Yamasaki, Naomi; Eklund, Mel; Jahn, Reinhard; Niemann, Heiner
CS Dep. Microbiol., Fed. Res. Cent. Virus Dis. Anim., Tuebingen, D-72001, Germany
SO Biochem. Biophys. Res. Commun. (1994), 200(2), 829-35
CODEN: BBRCA9; ISSN: 0006-291X
DT Journal
LA English
AB **Botulinum toxin** type G cleaves rat **synaptobrevin** 2 between Ala81 and Ala82, a peptide bond that differs from those attacked by tetanus **toxin** and the **botulinal** toxins types B, D, and F. **Synaptobrevin** isoforms carrying a Gly in the P1 position are poor substrates. Analyses of N-terminal deletion mutants of rat **synaptobrevin** 2 showed that a substrate starting at Leu54 is cleaved efficiently, whereas substrates beginning at Leu60 or Phe77 are cleaved partially or not at all, resp.

L24 ANSWER 42 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1995:59395 CAPLUS
DN 122:180311
TI Tetanus and **botulinum** neurotoxins are zinc proteases specific for proteins involved in vesicle docking and fusion
AU Schiavo, G.; Benfenati, F.; Poulain, B.; Rossetto, O.; Shone, C. C.; DasGupta, B. R.; Montecucco, C.
CS Dipartimento di Scienze Biomediche, Universita di Padova, Italy
SO Zentralbl. Bakteriol., Suppl. (1994), 24(Bacterial Protein Toxins), 375-85
CODEN: ZBASE2; ISSN: 0941-018X
DT Journal; General Review
LA English
AB A review with 32 refs. Tetanus and **botulinum** neurotoxins block the fusion of neurotransmitters or peptides contg. vesicles with the plasma membrane. We have shown that the light chains of these clostridial neurotoxins are intracellular enzymes. They are zinc-endoproteinases, whose activity is set free upon nicking of the single-chain **toxin** and redn. of the single interchain disulfide bond. Tetanus and **botulinum** B and F neurotoxins act specifically on **VAMP/synaptobrevin**, a membrane protein of the vesicles, which is cleaved at a single site. The single Gln-Phe peptide bond of **VAMP** is specifically cleaved by tetanus and **botulinum** B neurotoxin, while serotype F cleaves the single Gln-Lys bond of the sequence.

L24 ANSWER 43 OF 54 CAPLUS COPYRIGHT 1998 ACS
AN 1994:291492 CAPLUS
DN 120:291492
TI Inhibition of neurotransmitter release by tetanus and **botulinum** neurotoxins
AU Mochida, Sumiko

CS Dep. Physiol., Tokyo Med. Coll., Tokyo, 160, Japan
SO Seikagaku (1994), 66(3), 254-9
CODEN: SEIKAQ; ISSN: 0037-1017
DT Journal; General Review
LA Japanese
AB A review with 16 refs. on double-stranded structures, functions of each fragment, cloning of genes, identification of active sites, and functions as proteases in nerve ending of neurotoxins produced by Clostridium tetani and C. **botulinum**. Target mol. (e.g. **VAMP/synaptobrevin**, cellubrevin, SNAP-25, and syntaxin) of the neurotoxins are described.

L24 ANSWER 44 OF 54 MEDLINE
AN 95086179 MEDLINE
DN 95086179
TI Clostridial neurotoxins as tools to investigate the molecular events of neurotransmitter release.
AU Schiavo G; Rossetto O; Montecucco C
CS Dipartimento di Scienze Biomediche, Universit`a di Padova, Italy.
SO SEMINARS IN CELL BIOLOGY, (1994 Aug) 5 (4) 221-9. Ref: 74
Journal code: A60. ISSN: 1043-4682.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199503
AB The clostridial neurotoxins responsible for tetanus and botulism are eight different proteins, composed of two disulfide-linked polypeptide chains. They bind specifically to the presynaptic membrane via the heavy chain, while the light chain enters the cytosol of the neurons, where it displays a zinc-endopeptidase activity directed to proteins of the neuroexocytosis apparatus. Tetanus neurotoxin and **botulinum** neurotoxin serotypes B, D, F and G cleave specifically and at single different peptide bonds **VAMP/synaptobrevin**, a component of small synaptic vesicles. In contrast, the other neurotoxins catalyze the hydrolysis of proteins of the presynaptic membrane. Serotypes A and E of **botulinum** neurotoxin cleave SNAP-25, at different sites located within the carboxyl-terminus, while the specific target of serotype C is syntaxin.

L24 ANSWER 45 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 21
AN 94121241 EMBASE
TI Clostridial neurotoxins: New tools for dissecting exocytosis.
AU Niemann H.; Blasi J.; Jahn R.
CS Department of Microbiology, Federal Research Center for Viral, Diseases of Animals, Paul-Ehrlich-Str. 28, 72076 Tubingen, Germany, Federal Republic of
SO TRENDS CELL BIOL., (1994) 4/5 (179-185).
ISSN: 0962-8924 CODEN: TCBIEK
CY United Kingdom
DT Journal
FS 004 Microbiology
052 Toxicology
LA English
SL English
AB Tetanus **toxin** and **botulinal** toxins are potent inhibitors of neuronal exocytosis. Within the past five years the protein sequences of all eight neurotoxins have been determined, their mode of action as metalloproteases has been established, and their intraneuronal targets have been identified. The toxins act by selectively proteolysing the synaptic vesicle protein

synaptobrevin (**VAMP**) or the presynaptic membrane proteins syntaxin (HPC-1) and SNAP-25. These three proteins form the core of a complex that mediates fusion of carrier vesicles to target membranes. Tetanus and **botulinal** neurotoxins could serve in the future as tools to study membrane trafficking events, or even higher brain functions such as behaviour and learning.

L24 ANSWER 46 OF 54 MEDLINE DUPLICATE 22
AN 94377259 MEDLINE
DN 94377259
TI [Molecular mechanism of action of tetanus **toxin** and **botulinum** neurotoxins].
Mecanisme d'action moleculaire de la toxine tetanique et des neurotoxines botuliques.
AU Poulain B
CS Laboratoire de Neurobiologie Cellulaire et Moleculaire, CNRS, Gif-sur-yvette, France.
SO PATHOLOGIE BIOLOGIE, (1994 Feb) 42 (2) 173-82. Ref: 121
Journal code: OSG. ISSN: 0369-8114.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LA French
FS Priority Journals
EM 199412
AB Tetanus **toxin** and **botulinum** neurotoxins are di-chain proteins of 150 kD molecular weight. They are produced by bacteria of the Clostridium genus. These toxins act on the nervous system by inhibiting neurotransmitter release (glycine and GABA in the case of tetanus **toxin**; acetylcholine in the case of **botulinum** neurotoxins) thus inducing the spastic or flaccid paralysis that characterizes tetanus and botulism, respectively. Their cellular mechanism of action involves three main steps, namely binding to the neurone membrane, internalization and intracellular blockade of the release mechanism for neurotransmitters. Membrane acceptors for these toxins are not yet fully identified; they would consist of membrane gangliosides and proteins. The internalization step would be achieved by endocytosis. Recent findings show that both binding and internalization are mediated only by the heavy chain of the toxins whereas the intracellular blockade of neurotransmitter release involves their light chain alone. The light chain has been identified as a zinc metalloprotease and its substrates would be proteins involved in the neurotransmitter release mechanism. The target of tetanus **toxin** and of **botulinum** neurotoxin type B is **VAMP**/**synaptobrevin**, a membrane protein of the synaptic vesicles of nerve cell terminals.

L24 ANSWER 47 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 23
AN 94128625 EMBASE
TI Tetanus and **botulinum** neurotoxins are zinc proteases specific for components of the neuroexocytosis apparatus.
AU Schiavo G.; Rossetto O.; Benfenati F.; Poulain B.; Montecucco C.
CS Centro CNR Biomembrane, Dipartimento di Scienze, Biomed Sperimentali, Univ di Padova, 35121 Padova, Italy
SO ANN. NEW YORK ACAD. SCI., (1994) 710/- (65-75).
ISSN: 0077-8923 CODEN: ANYAA
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
052 Toxicology
LA English

SL English
AB Tetanus and **botulinum** neurotoxins bind to nerve cells, penetrate the cytosol, and block neurotransmitter release. Comparison of their amino-acid sequences shows the presence of the highly conserved His-Glu-x-x-His zinc-binding motif of zinc-endopeptidases (HExxH). Atomic absorption measurements of clostridial neurotoxins show the presence of one atom of zinc/ **toxin** molecule bound to the light chain. The **toxin** -bound zinc ion is essential for the neurotoxins inhibition of neurotransmitter release in Aplysia neurons injected with the toxins. Phosphoramidon, a very specific inhibitor of zinc-endopeptidases, blocks the intracellular activity of the clostridial neurotoxins. Highly purified preparations of the light chain of tetanus and **botulinum** B and F neurotoxins cleaved specifically **VAMP/synaptobrevin**, an integral membrane protein of small synaptic vesicles, both in vivo and in vitro. From these studies, it can be concluded that the clostridial neurotoxins responsible for tetanus and botulism block neuroexocytosis via the proteolytic cleavage of specific components of the neuroexocytotic machinery.

L24 ANSWER 48 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 24
AN 94213900 EMBASE
TI Mechanism of action of tetanus and **botulinum** neurotoxins.
AU Montecucco C.; Schiavo G.
CS Centro CNR Biomembrane, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO MOL. MICROBIOL., (1994) 13/1 (1-8).
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB The clostridial neurotoxins responsible for tetanus and botulism are metallo-proteases that enter nerve cells and block neurotransmitter release via zinc-dependent cleavage of protein components of the neuroexocytosis apparatus. Tetanus neurotoxin (TeNT) binds to the presynaptic membrane of the neuromuscular junction and is internalized and transported retroaxonally to the spinal cord. Whilst TeNT causes spastic paralysis by acting on the spinal inhibitory interneurons, the seven serotypes of **botulinum** neurotoxins (BoNT) induce a flaccid paralysis because they intoxicate the neuromuscular junction. TeNT and BoNT serotypes B, D, F and G specifically cleave **VAMP/synaptobrevin**, a membrane protein of small synaptic vesicles, at different single peptide bonds. Proteins of the presynaptic membrane are specifically attacked by the other BoNTs: serotypes A and E cleave SNAP-25 at two different sites located within the carboxyl terminus, whereas the specific target of serotype C is syntaxin.

L24 ANSWER 49 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 93335361 EMBASE
TI Identification of the nerve terminal targets of **botulinum** neurotoxin serotypes A, D, and E.
AU Schiavo G.; Rossetto O.; Catsicas S.; De Laureto P.P.; DasGupta B.R.; Benfenati F.; Montecucco C.
CS Dipartimento di Scienze Biomediche, CCNRB, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO J. BIOL. CHEM., (1993) 268/32 (23784-23787).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal

FS 029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB **Botulinum** neurotoxins are metalloproteins with one zinc atom bound to the zinc binding motif of zinc endopeptidases. Here we show that **botulinum** neurotoxin serotypes A, D, and E are zinc endoproteases specific for components of the synaptic vesicle docking and fusion complex. Serotypes A and E cleave SNAP-25, a 25-kDa protein of the synaptic terminal, while serotype D is specific for **VAMP/synaptobrevin**, a membrane protein of synaptic vesicles. Both rat brain **VAMP** isoforms are cleaved at a single Lys-Leu peptide bond. The proteolytic activity of these neurotoxins is inhibited by EDTA and captopril.

L24 ANSWER 50 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 25
AN 93168107 EMBASE
TI **Botulinum** neurotoxin serotype F is a zinc endopeptidase specific for **VAMP/synaptobrevin**.
AU Schiavo G.; Shone C.C.; Rossetto O.; Alexander F.C.G.; Montecucco C.
CS Dipartimento di Scienze Biomediche, CCNRB, Universita di Padova, Via Trieste 75, 35121 Padova, Italy
SO J. BIOL. CHEM., (1993) 268/16 (11516-11519).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB **Botulinum** neurotoxin serotype F contains the zinc binding motif of zinc endopeptidases. Atomic adsorption analysis of highly purified **toxin** preparation revealed the presence of one atom of zinc per molecule of **toxin**, which could be removed with EDTA or o-phenanthroline. The light chain of the neurotoxin was shown to have a zinc-dependent protease activity specific for **VAMP/synaptobrevin**, an integral membrane protein of synaptic vesicles. Both isoforms of rat **VAMP** were cleaved at the same site corresponding to the single Gln-Lys peptide bond present in their sequences. This proteolytic activity was inhibited by EDTA, o-phenanthroline, and captopril as well as by **VAMP** peptides spanning the cleavage site.

L24 ANSWER 51 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 26
AN 93241279 EMBASE
TI Antibodies against rat brain vesicle-associated membrane protein (**synaptobrevin**) prevent inhibition of acetylcholine release by tetanus **toxin** or **botulinum** neurotoxin type B.
AU Poulain B.; Rossetto O.; Deloye F.; Schiavo G.; Tauc L.; Montecucco C.
CS Lab. Neurobiologie Cellulaire/Molec., CNRS, F-91198 Gif-sur-Yvette, France
SO J. NEUROCHEM., (1993) 61/3 (1175-1178).
ISSN: 0022-3042 CODEN: JONRA
CY United States
DT Journal
FS 005 General Pathology and Pathological Anatomy
008 Neurology and Neurosurgery
052 Toxicology
LA English
SL English
AB Tetanus and **botulinum** B neurotoxins are zinc endopeptidases that cleave vesicle-associated membrane protein (

VAMP or **synaptobrevin**) at a single peptide bond. To test the possibility that in vivo also the **toxin** -induced blockade of neurotransmission is due to cleavage of **VAMP**, rat brain **VAMP**- specific antibodies were raised in rabbits. IgGs purified from one antiserum, which bind specifically to rat brain **VAMP**, also specifically recognize proteins from Aplysia californica in immunoblotting. When injected into neurons in the buccal ganglion of Aplysia, these IgGs did not affect the release of acetylcholine but effectively prevented the inhibitory action of both toxins on neurotransmitter release, thus indicating that the block of neurotransmission by these neurotoxins is consequent to the cleavage of **VAMP** or specific interaction with **VAMP**.

L24 ANSWER 52 OF 54 MEDLINE
AN 94054648 MEDLINE
DN 94054648
TI Tetanus and botulism neurotoxins: a new group of zinc proteases.
AU Montecucco C; Schiavo G
CS Department of Biomedical Sciences, University of Padova, Italy.
SO TRENDS IN BIOCHEMICAL SCIENCES, (1993 Sep) 18 (9) 324-7. Ref: 38
Journal code: WEF. ISSN: 0167-7640.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
EM 199402
AB The active forms of tetanus and **botulinum** neurotoxins, released from the precursor molecule by specific proteolysis and reduction, block the release of neurotransmitters via a Zn(2+)-dependent protease activity. **VAMP**/**synaptobrevin**, an integral membrane protein of the synaptic vesicles, is cleaved at a single site by tetanus and **botulinum** B, D and F neurotoxins. The unique sequence, mechanism of activation and site of activity of clostridial neurotoxins mark them out as an independent group of Zn(2+)-endopeptidases.

L24 ANSWER 53 OF 54 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 27
AN 93282075 EMBASE
TI **Botulinum** neurotoxin A selectively cleaves the synaptic protein SNAP-25.
AU Blasi J.; Chapman E.R.; Link E.; Binz T.; Yamasaki S.; De Camilli P.; Sudhof T.C.; Niemann H.; Jahn R.
CS Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University Medical School, POB 9812, New Haven, CT 06536, United States
SO NATURE, (1993) 365/6442 (160-163).
ISSN: 0028-0836 CODEN: NATUAS
CY United Kingdom
DT Journal
FS 004 Microbiology
029 Clinical Biochemistry
052 Toxicology
LA English
SL English
AB NEUROTRANSMITTER release is potently blocked by a group of structurally related **toxin** proteins produced by Clostridium **botulinum**. **Botulinum** neurotoxin type B (BoNT/B) and tetanus **toxin** (TeTx) are zinc-dependent proteases that specifically cleave **synaptobrevin** (**VAMP**), a membrane protein of synaptic vesicles. Here we report that inhibition of transmitter release from synaptosomes

caused by **botulinum** neurotoxin A (BoNT/A) is associated with the selective proteolysis of the synaptic protein SNAP-25. Furthermore, isolated or recombinant L chain of BoNT/A cleaves SNAP-25 in vitro. Cleavage occurred near the carboxyterminus and was sensitive to divalent cation chelators. In addition, a glutamate residue in the BoNT/A L chain, presumably required to stabilize a water molecule in the zinc-containing catalytic centre, was required for proteolytic activity. These findings demonstrate that BoNT/A acts as a zinc-dependent protease that selectively cleaves SNAP- 25. Thus, a second component of the putative fusion complex mediating synaptic vesicle exocytosis is targeted by a clostridial neurotoxin.

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=> d his

     (FILE 'HOME' ENTERED AT 08:57:05 ON 09 MAR 1998)

     FILE 'EMBASE, MEDLINE, BIOSIS, BIOTECHDS, LIFESCI, CONFSCI, WPIDS,
     JAPIO, DISSABS, CAPLUS' ENTERED AT 08:57:56 ON 09 MAR 1998
L1          271 S CELLUBREVIN
L2          108 S L1 AND (TETANUS OR BOTULINUM)
L3           18 S L2 AND NEUROTRANSMIT?
L4            8 DUP REM L3 (10 DUPLICATES REMOVED)
                E DOLLY JAMES OLIVER/AU
L5          109 S E1 OR E3
L6           62 S L5 AND TOXIN
L7           62 DUP REM L6 (0 DUPLICATES REMOVED)
L8           40 S L7 AND (CLOSTRID? OR BOTULIN? OR TETANUS)
L9            4 S L8 AND VAMP
L10           2 S L8 AND CELLUBREVIN
L11          12 S L8 AND NEUROMUSCULAR
                E AOKI KEI ROGER/AU
L12           5 S E3
                E WHEELER LARRY ALLEN/AU
L13          35 S E2 OR E3
L14           0 S L13 AND NEUROTOXIN
L15           0 S L13 AND CELLUBREVIN
L16           2 S L13 AND TOXIN
L17           0 S L13 AND VAMP
                E GARST MICHAEL ELWOOD/AU
L18          81 S E2 OR E3
L19           2 S L18 AND TOXIN
L20           0 S L18 AND CELLUBREVIN
L21         477 S VAMP AND SYNAPTOBREVIN
L22         192 S L21 AND TOXIN
L23         128 S L22 AND BOTULIN?
L24          54 DUP REM L23 (74 DUPLICATES REMOVED)

=> s l1 and l21

L25          68 L1 AND L21

=> dup rem l25

PROCESSING COMPLETED FOR L25
L26          25 DUP REM L25 (43 DUPLICATES REMOVED)

=> d bib ab 1-25


L26  ANSWER 1 OF 25  EMBASE  COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN   97123645  EMBASE
TI   Syntaxin 4, VAMP2, and/or VAMP3/cellubrevin are functional
     target membrane and vesicle SNAP receptors for insulin-stimulated
     GLUT4 translocation in adipocytes.
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AU Olson A.L.; Knight J.B.; Pessin J.E.
CS United States. Jeffrey-Pessin@UIOWA.EDU
SO Molecular and Cellular Biology, (1997) 17/5 (2425-2435).
Refs: 63
ISSN: 0270-7306 CODEN: MCEBD4
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Introduction of the cytoplasmic domain of syntaxin 4, using either recombinant vaccinia virus or single-cell microinjection, resulted in an inhibition of insulin-stimulated GLUT4 but not GLUTI translocation to the plasma membrane. This was specific for syntaxin 4, since neither the expression of syntaxin 3 nor the expression of a syntaxin 4 mutant in which the vesicle-associated membrane protein (**VAMP**) binding site was deleted had any significant effect. Consistent with the requirement for a functional **VAMP** binding site, expression of the cytoplasmic domains of VAMP2 or VAMP3/**cellubrevin** also resulted in an inhibition of insulin-stimulated GLUT4 translocation. In addition, immunoprecipitation of the expressed syntaxin 4 cytoplasmic domain resulted in an insulin-stimulated increase in the coimmunoprecipitation of GLUT4-containing vesicles. Together, these data demonstrate that syntaxin 4, VAMP2, and/or VAMP3/**cellubrevin** can function as target membrane and vesicle SNAP receptors, respectively, for insulin- responsive GLUT4 translocation to the plasma membrane.

L26 ANSWER 2 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97153176 EMBASE
TI Association of N-ethylmaleimide sensitive fusion (NSF) protein and soluble NSF attachment proteins-.alpha. and -.gamma. with glucose transporter-4- containing vesicles in primary rat adipocytes.
AU Mastick C.C.; Falick A.L.
CS C.C. Mastick, Parke-Davis Pharmaceut. Res. Div., Warner-Lambert Company, Ann Arbor, MI 48105, United States. masticc@aa.wl.com
SO Endocrinology, (1997) 138/6 (2391-2397).
Refs: 46
ISSN: 0013-7227 CODEN: ENDOAO
CY United States
DT Journal
FS 003 Endocrinology
029 Clinical Biochemistry
LA English
SL English
AB To investigate the role of N-ethylmaleimide sensitive fusion protein (NSF) and soluble NSF attachment proteins (SNAP)-containing fusion complexes in glucose transporter-4 (GLUT4) membrane trafficking, the subcellular distributions of NSF, .alpha.-SNAP, and .gamma.-SNAP in primary rat adipocytes were determined. A large fraction of the NSF and SNAPs were associated with intracellular membranes, distributed between the low-density microsomes (LDM) and high-density microsomes. Very little of the NSF and SNAPs were associated with the plasma membrane fraction. This distribution did not change after insulin stimulation. Approximately 75% of the NSF and SNAPs in the LDM fraction were coimmunoprecipitated with 85% of the GLUT4 and 60% of the vesicle associated membrane proteins (VAMPs; synaptobrevins) **VAMP**-2 and **cellubrevin** in anti-GLUT4 immunoadsorptions. In contrast to NSF and the SNAPs, the .beta.-coatomer protein (.beta.-COP) found in the LDM fraction was excluded from GLUT4 vesicles. When LDM fractions were solubilized with Thesit (octaethylene glycol dodecyl ether) or Triton X-100, approximately 40% of the .alpha.-SNAP was colocalized with NSF on glycerol gradients in large (.apprx.20S), ATP- sensitive complexes.

VAMP-2 and **cellubrevin** are concentrated in the LDM fractions and in GLUT4 vesicles; both were excluded from these complexes. These data suggest that the steady state association of NSF and the SNAPs with GLUT4 vesicles and cell membranes is independent of the formation of fusion complexes.

L26 ANSWER 3 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97022925 EMBASE
TI Proteolytic cleavage of **cellubrevin** and vesicle-associated membrane protein (**VAMP**) by tetanus toxin does not impair insulin-stimulated glucose transport or GLUT4 translocation in rat adipocytes.
AU Hajduch E.; Aledo J.C.; Watts C.; Hundal H.S.
CS United Kingdom
SO Biochemical Journal, (1997) 321/1 (233-238).
Refs: 41
ISSN: 0264-6021 CODEN: BIJOAK
CY United Kingdom
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Acute insulin stimulation of glucose transport in fat and skeletal muscle occurs principally as a result of the hormonal induced translocation of the GLUT4 glucose transporter from intracellular vesicular stores to the plasma membrane. The precise mechanisms governing the fusion of GLUT4 vesicles with the plasma membrane are very poorly understood at present but may share some similarities with synaptic vesicle fusion, as vesicle-associated membrane protein (**VAMP**) and **cellubrevin**, two proteins implicated in the. process of membrane fusion, are resident in GLUT4-containing vesicles isolated from rat and murine 3T3-L1 adipocytes respectively. In this study we show that proteolysis of both **cellubrevin** and **VAMP**, induced by electroporation of isolated rat adipocytes with tetanus toxin, does not impair insulin-stimulated glucose transport or GLUT4 translocation. The hormone was found to stimulate glucose uptake by approx. 16-fold in freshly isolated rat adipocytes. After a single electroporating pulse, the ability of insulin to activate glucose uptake was lowered, but the observed stimulation was nevertheless nearly 5-fold higher than the basal rate of glucose uptake. Electroporation of adipocytes with 600 nM tetanus toxin resulted in a complete loss of both **cellubrevin** and **VAMP** expression within 60 min. However, toxin-mediated proteolysis of both these proteins had no effect on the ability of insulin to stimulate glucose transport which was elevated approx. 5-fold, an activation of comparable magnitude to that observed in cells electroporated without tetanus toxin. The lack of any significant change in insulin-stimulated glucose transport was consistent with the finding that toxin-mediated proteolysis of both **cellubrevin** and **VAMP** had no detectable effect on insulin-induced translocation of GLUT4 in adipocytes. Our findings indicate that, although **cellubrevin** and **VAMP** are resident proteins in adipocyte GLUT4-containing vesicles, they are not required for the acute insulin-induced delivery of GLUT4 to the plasma membrane.

L26 ANSWER 4 OF 25 CAPLUS COPYRIGHT 1998 ACS
AN 1997:691089 CAPLUS
DN 128:31019
TI Tissue-specific alternative RNA splicing of rat vesicle-associated membrane protein-1 (**VAMP**-1)
AU Mandic, Robert; Trimble, William S.; Lowe, Anson W.
CS Department of Medicine and the Digestive Disease Center, Stanford University School of Medicine, Stanford, USA

SO Gene (1997), 199(1-2), 173-179
CODEN: GENED6; ISSN: 0378-1119
PB Elsevier
DT Journal
LA English
AB The vesicle-assocd. membrane protein (**VAMP**) family is essential to vesicle-mediated protein transport. Three mammalian isoforms, **VAMP**-1, **VAMP**-2, and **cellubrevin**, play a role in protein transport to the plasma membrane. In this study, we describe a new rat **VAMP**-1 isoform produced by alternative pre-mRNA splicing. Only one **VAMP**-1 isoform dominates in each tissue. Anal. of the nucleotide sequence for the newly discovered isoform, **VAMP** -1b, reveals that its expression is detd. by whether an intron is retained or removed. The predicted amino acid sequences for the **VAMP**-1 isoforms differ at the carboxy-terminal end of the protein. A similar process has been described for VAMPs in Drosophila melanogaster and suggests a conserved function for the carboxy-terminal domain that can be modulated.

L26 ANSWER 5 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97009114 EMBASE
TI Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins.
AU Cheatham B.; Volchuk A.; Kahn C.R.; Wang L.; Rhodes C.J.; Klip A.
CS United States. cheathab@joslab.harvard.edu
SO Proceedings of the National Academy of Sciences of the United States of America, (1996) 93/26 (15169-15173).
Refs: 32
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB A major physiological role of insulin is the regulation of glucose uptake into skeletal and cardiac muscle and adipose tissue, mediated by an insulin-stimulated translocation of GLUT4 glucose transporters from an intracellular vesicular pool to the plasma membrane. This process is similar to the regulated docking and fusion of vesicles in neuroendocrine cells, a process that involves SNARE-complex proteins. Recently, several SNARE proteins were found in adipocytes: vesicle-associated membrane protein (**VAMP**- 2), its related homologue **cellubrevin**, and syntaxin-4. In this report we show that treatment of permeabilized 3T3-L1 adipocytes with botulinum neurotoxin D, which selectively cleaves **VAMP**-2 and **cellubrevin**, inhibited the ability of insulin to stimulate translocation of GLUT4 vesicles to the plasma membrane. Furthermore, treatment of the permeabilized adipocytes with glutathione S- transferase fusion proteins encoding soluble forms of **VAMP**-2 or syntaxin-4 also effectively blocked insulin-regulated GLUT4 translocation. These results provide evidence of a functional role for SNARE-complex proteins in insulin-stimulated glucose uptake and suggest that adipocytes utilize a mechanism of regulating vesicle docking and fusion analogous to that found in neuroendocrine tissues.

L26 ANSWER 6 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97016928 EMBASE
TI Mutational analysis of **VAMP** domains implicated in Ca2+-induced insulin exocytosis.
AU Regazzi R.; Sadoul K.; Meda P.; Kelly R.B.; Halban P.A.; Wollheim C.B.
CS R. Regazzi, Division de Biochimie Clinique, Departement de Medicine Interne, Universite de Geneve, CH-1211 Geneve 4, Switzerland

SO EMBO Journal, (1996) 15/24 (6951-6959).
Refs: 40
ISSN: 0261-4189 CODEN: EMJODG
CY United Kingdom
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Vesicle-associated membrane protein-2 (**VAMP**-2) and **cellubrevin** are associated with the membrane of insulin-containing secretory granules and of .gamma.-aminobutyric acid (GABA)-containing synaptic-like vesicles of pancreatic .beta.-cells. We found that a point mutation in **VAMP**-2 preventing targeting to synaptic vesicles also impairs the localization on insulin-containing secretory granules, suggesting a similar requirement for vesicular targeting. Tetanus toxin (TeTx) treatment of permeabilized HIT-T15 cells leads to the proteolytic cleavage of **VAMP**-2 and **cellubrevin** and causes the inhibition of Ca2+-triggered insulin exocytosis. Transient transfection of HIT-T15 cells with **VAMP**-1, **VAMP** -2 or **cellubrevin** made resistant to the proteolytic action of TeTx by amino acid replacements in the cleavage site restored Ca2+-stimulated secretion. Wild-type **VAMP**-2, wild-type **cellubrevin** or a mutant of **VAMP**-2 resistant to TeTx but not targeted to secretory granules were unable to rescue Ca2+-evoked insulin release. The transmembrane domain and the N-terminal region of **VAMP**-2 were not essential for the recovery of stimulated exocytosis, but deletions preventing the binding to SNAP-25 and/or to syntaxin I rendered the protein inactive in the reconstitution assay. Mutations of putative phosphorylation sites or of negatively charged amino acids in the SNARE motif recognized by clostridial toxins had no effect on the ability of **VAMP**-2 to mediate Ca2+-triggered secretion. We conclude that: (i) both **VAMP**-2 and **cellubrevin** can participate in the exocytosis of insulin; (ii) the interaction of **VAMP**-2 with syntaxin and SNAP-25 is required for docking and/or fusion of secretory granules with the plasma membrane; and (iii) the phosphorylation of **VAMP**-2 is not essential for Ca2+ stimulated insulin exocytosis.

L26 ANSWER 7 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 97000358 EMBASE
TI Molecular components of the exocytotic machinery in the rat pituitary gland.
AU Jacobsson G.; Meister B.
CS Dr. B. Meister, Berzelius Laboratory, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden
SO Endocrinology, (1996) 137/12 (5344-5356).
ISSN: 0013-7227 CODEN: ENDOAO
CY United States
DT Journal
FS 003 Endocrinology
029 Clinical Biochemistry
LA English
SL English
AB Several protein components that are essential for exocytotic membrane fusion in neurons have recently been identified. The expression and cellular localization of such protein components were examined in the rat pituitary gland. In situ hybridization using isoform-specific oligonucleotide probes to different exocytotic protein messenger RNAs (mRNAs) showed strong hybridization signal for synaptotagmin-I, cysteine string protein (CSP), **VAMP**-2 (vesicle-associated membrane protein), **cellubrevin**, munc-18 (mammalian homologue of unc-18), SNAP-25a (synaptosomal-associated protein of 25 kDa), syntaxin 1A, syntaxin

4, syntaxin 5, and .alpha.-SNAP (soluble NSF attachment protein) in the anterior and intermediate, but not in the posterior lobe of the pituitary. Moderate to weak hybridization signal was detected for synaptotagmin III. SNAP-25b, and syntaxin 2 mRNA in the anterior and intermediate, but not in the posterior lobe of the pituitary. Synaptotagmin II, **VAMP**-I, syntaxin 1B, or syntaxin 3 mRNA expression could not be detected in any part of the pituitary gland. Immunofluorescence histochemistry in combination with confocal laser microscopy revealed that synaptotagmin-, **VAMP**-, CSP-, NSF-, and .alpha.-SNAP-like immunoreactivities (-LI) were present in granules of cells in the anterior and intermediate lobe, whereas SNAP-25 and syntaxin-LI were primarily located to the plasma membrane. Synaptotagmin-, **VAMP**-, CSP-, NSF-, .alpha.-SNAP-, SNAP-25- and syntaxin-LI were all present in nerve fibers of the posterior lobe. Within cells of the anterior lobe, colocalization could be demonstrated for synaptotagmin I/II- and synaptotagmin III-LI with ACTH-, GH-, PRL- and TSH-, but not FSH- or LH-LI, whereas **VAMP**, CSP-, NSF-, .alpha.-SNAP-, SNAP-25 and syntaxin-LI were demonstrated in all hormone-containing cell types of the anterior pituitary. The results show the presence of several protein components and their isoform-specific mRNAs in the rat pituitary gland, suggesting that these proteins, similar to their roles in regulation of synaptic neurotransmitter release, may participate in exocytotic events in endocrine pituitary cells and in neurosecretory nerve endings of the neurohypophysis.

L26 ANSWER 8 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 96369083 EMBASE
TI The vesicle-associated membrane protein family of proteins in rat pancreatic and parotid acinar cells.
AU Gaisano H.Y.; Sheu L.; Grondin G.; Ghai M.; Bouquillon A.; Lowe A.; Beaudoin A.; Trimble W.S.
CS Medical Sciences Building, University of Toronto, Toronto, Ont. M5S 1A8, Canada
SO Gastroenterology, (1996) 111/6 (1661-1669).
ISSN: 0016-5085 CODEN: GASTAB
CY United States
DT Journal
FS 029 Clinical Biochemistry
048 Gastroenterology
LA English
SL English
AB Background and Aims: The vesicle-associated membrane protein (**VAMP**) family of proteins may play an important role in regulating enzyme secretion from pancreatic and parotid acini. The purpose of this study was to characterize the isoforms produced in pancreatic and parotid acini and determine their subcellular locations. Methods: Using a battery of specific antisera and recombinant tetanus toxin light chain (which cleaves **VAMP** -2 and **cellubrevin**), the presence of each **VAMP** molecule in the acini was determined by immunoblotting of subcellular membrane fractions; their localization was determined by confocal immunofluorescence microscopy and immunogold electron microscopy. Results: Both **VAMP**-2 and **cellubrevin** were present on both the zymogen granule membrane and plasma membrane. **VAMP**-1 was not present in the acinar cell but was found in the nerve endings innervating the acini. As expected, pancreatic acinar **VAMP**-2 and **cellubrevin** were sensitive to cleavage by recombinant tetanus toxin. Conclusions: **VAMP**-2 and **cellubrevin** may play integral roles in exocytosis of the pancreatic and parotid acinar cells, whereas **VAMP**-1 is restricted to nerves that innervate the acini and may function to modulate exocrine activity.

L26 ANSWER 9 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 1

AN 96222229 EMBASE
TI Syntaxin 4 in 3T3-L1 adipocytes: Regulation by insulin and participation in insulin-dependent glucose transport.
AU Volchuk A.; Wang Q.; Ewart H.S.; Liu Z.; He L.; Bennett M.K.; Klip A.
CS Division of Cell Biology, Hospital for Sick Children, 555 University Avenue, Toronto, Ont. M5G 1X8, Canada
SO Molecular Biology of the Cell, (1996) 7/7 (1075-1082).
ISSN: 1059-1524 CODEN: MBCEEV
CY United States
DT Journal
FS 002 Physiology
029 Clinical Biochemistry
LA English
SL English
AB Syntaxins are thought to be membrane receptors that bind proteins of the **synaptobrevin**/vesicle-associated membrane protein (**VAMP**) family found on transport vesicles. Recently, we detected **synaptobrevin** II and **cellubrevin** on immunopurified vesicles containing the glucose transporter 4 (GLUT4) in insulin-responsive cells. In an effort to identify the plasma membrane receptors for these vesicles, we now examine the expression of syntaxins in the 3T3-L1 adipocyte cell line. Neither syntaxin 1A nor 1B was found, in keeping with the neuronal restriction of these isoforms. In contrast, syntaxins 2 and 4 were readily detectable. By subcellular fractionation and estimation of protein yields, 67% of syntaxin 4 was localized to the plasma membrane, 24% to the low-density microsomes, and 9% to the high-density microsomes. Interestingly, acute insulin treatment decreased the content of syntaxin 4 in low-density microsomes and caused a corresponding gain in the plasma membrane fraction, reminiscent of the recruitment of GLUT4 glucose transporters. In contrast, there was no change in the distribution of syntaxin 2, which was mostly associated in the plasma membrane. A fraction of the intracellular syntaxin 4 was recovered with immunopurified GLUT4- containing vesicles. Moreover, anti-syntaxin 4 antibodies introduced into permeabilized 3T3-L1 adipocytes significantly reduced the insulin-dependent stimulation of glucose transport, in contrast to the introduction of irrelevant immunoglobulin G, which was without consequence. We propose that either the plasma membrane and/or the vesicular syntaxin 4 are involved in docking and/or fusion of GLUT4 vesicles at the cell surface of 3T3-L1 adipocytes.

L26 ANSWER 10 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 2
AN 96202254 EMBASE
TI Cleavage of vesicle-associated membrane protein (**VAMP**)-2 and **cellubrevin** on GLUT4-containing vesicles inhibits the translocation of GLUT4 in 3T3-L1 adipocytes.
AU Tamori Y.; Hashiramoto M.; Araki S.; Kamata Y.; Takahashi M.; Kozaki S.; Kasuga M.
CS Centre Molecular Cellular Biology, University of Queensland, Brisbane, QLD 4072, Australia
SO Biochemical and Biophysical Research Communications, (1996) 220/3 (740-745).
ISSN: 0006-291X CODEN: BBRCA
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB We have identified **VAMP** isoforms, **VAMP**-2 and **cellubrevin**, on GLUT4-containing vesicle membranes isolated from 3T3-L1 adipocytes. These proteins translocate from a low density microsomal fraction to the plasma membrane upon insulin

stimulation in a fashion similar to GLUT4. **VAMP**-1 was not detected in this low density microsomal fraction nor on purified GLUT4-containing vesicles. In streptolysin-O permeabilized 3T3-L1 adipocytes, both **VAMP**-2 and **cellubrevin** were cleaved with botulinum neurotoxin isoform B, BoNTx/B. In addition, BoNTx/B partially inhibited insulin-stimulated GLUT4 translocation and glucose transport activity. We conclude that the **synaptobrevin** isoforms are important components of the insulin-dependent translocation of GLUT4 to the cell surface in adipocytes.

L26 ANSWER 11 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 3
AN 96247934 EMBASE
TI The glucose transporter (GLUT-4) and vesicle-associated membrane protein-2 (**VAMP**-2) are segregated from recycling endosomes in insulin- sensitive cells.
AU Martin S.; Tellam J.; Livingstone C.; Slot J.W.; Gould G.W.; James D.E.
CS Molecular/Cellular Biology Center, University of Queensland, St. Lucia, Brisbane, QLD 4072, Australia
SO Journal of Cell Biology, (1996) 134/3 (625-635).
ISSN: 0021-9525 CODEN: JCLBA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Insulin stimulates glucose transport in adipocytes by translocation of the glucose transporter (GLUT-4) from an intracellular site to the cell surface. We have characterized different **synaptobrevin**/vesicle-associated membrane protein (**VAMP**) homologues in adipocytes and studied their intracellular distribution with respect to GLUT-4. **VAMP**-1, **VAMP**-2, and **cellubrevin** cDNAs were isolated from a 3T3-L1 adipocyte expression library. **VAMP**-2 and **cellubrevin** were: (a) the most abundant isoforms in adipocytes, (b) detectable in all insulin responsive tissues, (c) translocated to the cell surface in response to insulin, and (d) found in immunoadsorbed GLUT-4 vesicles. To further define their intracellular distribution, 3T3-L1 adipocytes were incubated with a transferrin/HRP conjugate (Tf/HRP) and endosomes ablated following addition of DAB and H2O2. While this resulted in ablation of >90% of the transferrin receptor (TfR) and **cellubrevin** found in intracellular membranes, 60% of GLUT-4 and 90% of **VAMP**-2 was not ablated. Immuno-EM on intracellular vesicles from adipocytes revealed that **VAMP**-2 was colocalized with GLUT-4, whereas only partial colocalization was observed between GLUT-4 and **cellubrevin**. These studies show that two different v-SNAREs, **cellubrevin** and **VAMP**-2, are partially segregated in different intracellular compartments in adipocytes, implying that they may define separate classes of secretory vesicles in these cells. We conclude that a proportion of GLUT-4 is found in recycling endosomes in nonstimulated adipocytes together with **cellubrevin** and the transferrin receptor. In addition, GLUT-4 and **VAMP**-2 are selectively enriched in a postendocytic compartment. Further study is required to elucidate the function of this latter compartment in insulin-responsive cells.

L26 ANSWER 12 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 4
AN 96377645 EMBASE
TI Identification of SNAP receptors in rat adipose cell membrane fractions and in SNARE complexes co-immunoprecipitated with epitope-tagged N-ethylmaleimide-sensitive fusion protein.

AU Timmers K.I.; Clark A.E.; Omatsu-Kanbe M.; Whiteheart S.W.; Bennett M.K.; Holman G.D.; Cushman S.V.

CS Lab. Theoretical Physical Biology, Nat. Inst. Child Health Human Dev., Building 10, National Insts. Health, 10 Center Drive MSC 1855, Bethesda, MD 20892-1855, United States

SO Biochemical Journal, (1996) 320/2 (429-436).
ISSN: 0264-6021 CODEN: BIJOAK

CY United Kingdom

DT Journal

FS 029 Clinical Biochemistry

LA English

SL English

AB The vesicle-associated membrane proteins [VAMPs; vesicle SNAP receptors (v-SNAREs)] present on GLUT4-enriched vesicles prepared from rat adipose cells have been identified as **synaptobrevin** 2 (**VAMP** 2) and **cellubrevin** (**VAMP** 3) by using isoform-specific antisera. Additional antisera identify syntaxins 2 and 4 as the predominant target membrane SNAP receptors (t-SNAREs) in the plasma membranes (PM), with syntaxin 3 at one-twentieth the level. Syntaxins 2 and 4 are enriched 5-10-fold in PM compared with low-density microsomes (LDM). Insulin treatment results in an 11-fold increase in immunodetectable GLUT4 in PM and smaller (approx. 2-fold) increases in **VAMP** 2 and **VAMP** 3, whereas the subcellular distributions of the syntaxins are not altered by insulin treatment. To determine which of the SNAP receptors (SNAREs) in PM might participate in SNARE complexes with proteins from GLUT4 vesicles, complexes were immunoprecipitated with anti-myc antibody from solubilized membranes after the addition of myc-epitope-tagged N-ethylmaleimide-sensitive fusion protein (NSF) and recombinant .alpha.-soluble NSF attachment protein (.alpha.SNAP). These complexes contain VAMPs 2 and 3 and syntaxin 4, but not syntaxins 2 or 3. Complex formation requires ATP and is disrupted by ATP hydrolysis. When all membrane fractions are prepared from basal cells, few or no VAMPs and no syntaxin 4 are immunoprecipitated in SNARE complexes obtained from LDM alone (or from immunoisolated GLUT4 vesicles). The content of syntaxin 4 depends on the presence of PM, and participation of VAMPs 2 and 3 is enhanced 4-6-fold by the addition of solubilized GLUTL4 vesicles to PM. The latter increase is greater than can be explained by the 2-fold higher levels of VAMPs added to the reaction mixture. When all membrane fractions are prepared from insulin-stimulated cells, SNARE complexes formed from PM alone contain similar levels of syntaxin 4 but 5-6-fold higher levels of VAMPs 2 and 3 compared with PM alone from basal cells. Addition of GLUT4 vesicle proteins to PM from insulin-treated cells results in a further 2-fold increase in **VAMP** 2 recovered in SNARE complexes. Therefore the VAMPs in PM of insulin-treated but not basal cells, and in GLUT4-vesicles from cells in either condition, are in a form that readily forms a SNARE complex with PM t-SNAREs and NSF. Insulin seems to activate PM and/or GLUT4 vesicles so as to increase the efficiency of SNARE complex formation.

L26 ANSWER 13 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 5

AN 96247031 EMBASE

TI Localization of **cellubrevin** to the Golgi complex in pancreatic acinar cells.

AU Sengupta D.; Gumkowski F.D.; Tang L.H.; Chilcote T.J.; Jamieson J.D.

CS Department Cell Biology, SHM C 215, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, United States

SO European Journal of Cell Biology, (1996) 70/4 (306-314).
ISSN: 0171-9335 CODEN: EJCBDN

CY Germany, Federal Republic of

DT Journal

FS 029 Clinical Biochemistry

LA English
SL English
AB **Cellubrevin** is the smallest (14 kDa) isoform of the **synaptobrevin** (**VAMP**) protein family and is found in a wide variety of tissues. Western blot analysis with a polyclonal antibody against the unique N-terminus of **cellubrevin** identified a protein of 14 kDa in rat pancreas. This protein distributed predominantly to the particulate fractions from the rat exocrine pancreas and was totally resistant to NaHCO3 washes, indicating that it is an integral membrane protein. Subcellular fractionation of pancreatic homogenates showed enrichment of this protein in the smooth microsomal fraction while negligible amounts were present in the zymogen granule membrane or the rough microsomal membrane fractions. As seen in other tissues, the 14 kDa immunoreactive form was proteolyzed by tetanus toxin. Light and electron microscopic immunocytochemistry localized **cellubrevin** immunoreactivity primarily to small vesicles and condensing vacuoles originating from the Golgi region, with significantly lower labeling on zymogen granules. Based on the intracellular localization of **cellubrevin** detected in acinar cells by immunocytochemistry and cell fractionation, we suggest that **cellubrevin** may be involved in the maturation of secretory granules.

L26 ANSWER 14 OF 25 CAPLUS COPYRIGHT 1998 ACS
AN 1996:308679 CAPLUS
DN 125:1470
TI The glucose transporters of skeletal muscle
AU Klip, Amira; Volchuk, Allen; He, Lijing; Tsakiridis, Theodoros
CS Hospital Sick Children, 555 University Avenue, Toronto, ON, M5G 1X8, Can.
SO Semin. Cell Dev. Biol. (1996), 7(2), 229-237
CODEN: SCDBFX; ISSN: 1084-9521
DT Journal; General Review
LA English
AB A review with 72 refs. Glucose transport into skeletal muscle occurs through the GLUT1 and GLUT4 glucose transporters. Muscle cells in culture also express the GLUT3 fetal muscle/neuronal type transporter. In skeletal muscle, the GLUT1 transporter is restricted to the cell surface, while the more abundant GLUT4 transporter is largely sequestered intracellularly from where it is rapidly translocated to the cell surface in response to insulin, exercise or hypoxia. The insulin effect has been documented by subcellular fractionation of rat, mouse and human muscle, and has been confirmed quant. by photolabeling of the surface transporters and qual. by immunoelectron microscopy. In L6 myotubes in culture, the GLUT1 and GLUT3 transporters are mostly located at the cell surface but a fraction resides intracellularly, whereas the GLUT4 transporter is distributed evenly between the surface and the intracellular location. Immunopurified intracellular GLUT4 vesicles from these cells do not contain appreciable amts. of GLUT1 or GLUT3 transporters, although all three transporters respond to insulin by translocating to the plasma membrane. The glucose transporter translocation induced by insulin in skeletal muscle and L6 myotubes requires phosphatidylinositol 3-kinase activity, as does the maintenance of the basal amt. of transporters at the plasma membrane. Two different phosphatidylinositol 3-kinase activities may control basal and insulin-dependent transport. In contrast, the stimulation of glucose transport induced by exercise or hypoxia is independent of this enzymic activity. In both L6 myotubes and mature skeletal muscle, the GLUT4-contg. vesicle contains **synaptobrevin** II/**VAMP**-2 and **cellubrevin**. These proteins also redistribute in response to insulin, and may be required for correct vesicle docking and/or fusion with the plasma membrane.

L26 ANSWER 15 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 6
AN 95116829 EMBASE
TI **Cellubrevin** is a resident protein of insulin-sensitive GLUT4 glucose transporter vesicles in 3T3-L1 adipocytes.
AU Volchuk A.; Sargeant R.; Sumitani S.; Liu Z.; He L.; Klip A.
CS Div. of Cell Biology, 555 University Ave., Toronto, Ont. M5G 1X8, Canada
SO Journal of Biological Chemistry, (1995) 270/14 (8233-8240). ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB Insulin stimulates glucose transport in muscle and fat cells by inducing translocation of GLUT4 glucose transporters from a storage site to the cell surface. The mechanism of this translocation and the identity of the storage site are unknown, but it has been hypothesized that transporters recycle between an insulin-sensitive pool, endosomes, and the cell surface. Upon cell homogenization and fractionation, the storage site migrates with light microsomes (LDM) separate from the plasma membrane fraction (PM). **Cellubrevin** is a recently identified endosomal protein that may be involved in the reexocytosis of recycling endosomes. Here we describe that **cellubrevin** is expressed in 3T3-L1 adipocytes and is more abundant in the LDM than in the PM. **Cellubrevin** was markedly induced during differentiation of 3T3-L1 fibroblasts into adipocytes, in parallel with GLUT4, and the development of insulin regulated traffic. In response to insulin, the **cellubrevin** content decreased in the LDM and increased in the PM, suggesting translocation akin to that of the GLUT4 glucose transporter. Vesicle-associated membrane protein 2 (**VAMP**-2)/**synaptobrevin**-II, a protein associated with regulated exocytosis in secretory cells, also redistributed in response to insulin. Both **cellubrevin** and **VAMP**-2 were susceptible to cleavage by tetanus toxin. Immunopurified GLUT4-containing vesicles contained **cellubrevin** and **VAMP**-2, and immunopurifled **cellubrevin**-containing vesicles contained GLUT4 protein, but undiscernible amounts of **VAMP**-2. These observations suggest that **cellubrevin** and **VAMP**-2 are constituents of the insulin-regulated pathway of membrane traffic. These results are the first demonstration that **cellubrevin** is present in a regulated intracellular compartment. We hypothesize that **cellubrevin** and **VAMP**-2 may be present in different subsets of GLUT4-containing vesicles.

L26 ANSWER 16 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.
AN 95366414 EMBASE
TI Subcellular distribution of docking/fusion proteins in neutrophils, secretory cells with multiple exocytic compartments.
AU Brumell J.H.; Volchuk A.; Sengelov H.; Borregaard N.; Cieutat A.-M.; Bainton D.F.; Grinstein S.; Klip A.
CS Division of Cell Biology, Hospital for Sick Children, 555 University Avenue, Toronto, Ont. M5G-1X8, Canada
SO Journal of Immunology, (1995) 155/12 (5750-5759). ISSN: 0022-1767 CODEN: JOIMA3
CY United States
DT Journal
FS 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LA English
SL English

AB Neutrophils contain at least four distinct types of secretory organelles, which undergo exocytosis during infection and inflammation. The signaling pathways leading to secretion of individual granules and their kinetics of exocytosis vary greatly, causing temporal and regional differences in docking and fusion with the plasma membrane. As a step toward understanding the processes underlying differential granular secretion in neutrophils, we assessed the presence and distribution of a number of proteins reported to be involved in vesicular docking and/or fusion in other systems. Specific Abs were used for immunoblotting of cells fractionated by density gradients and free-flow electrophoresis, and for localization by confocal immunofluorescence and electron microscopy. Syntaxin 1, **VAMP** (vesicle- associated membrane protein)-1, synaptosome-associated protein-25 (SNAP-25), synaptophysin, and **cellubrevin** were not detectable in human neutrophils. In contrast, syntaxin 4, **VAMP**-2, and the 39-kDa isoform of secretory carrier membrane protein (SCAMP) were present. SCAMP was found mainly in secondary and tertiary granules and in a fraction containing secretory vesicles, but was virtually absent from the primary (lysosomal) granules. This profile is consistent with the proposed 'post-Golgi' distribution of SCAMP. **VAMP**-2 was largely absent from primary and secondary granules, but concentrated in tertiary granules and secretory vesicles. This pattern of distribution parallels the increasing sensitivity of these exocytic compartments to intracellular free calcium. Accordingly, ionomycin induced translocation of **VAMP**-2 toward the plasma membrane. Syntaxin 4 was found almost exclusively in the plasma membrane, and it accumulated in lamellipodia of migrating cells. This regional accumulation may contribute to localized secretion into the phagosomal lumen.

L26 ANSWER 17 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 7

AN 95337722 EMBASE

TI A survey of GTP-binding proteins and other potential key regulators of exocytotic secretion in eosinophils. Apparent absence of rab3 and vesicle fusion protein homologues.

AU Lacy P.; Thompson N.; Tian R.; Solari R.; Hide I.; Newman T.M.; Gomperts B.D.

CS Department of Physiology, University College London, University Street, London, United Kingdom

SO Journal of Cell Science, (1995) 108/11 (3547-3556).
ISSN: 0021-9533 CODEN: JNCSAI

CY United Kingdom

DT Journal

FS 029 Clinical Biochemistry

LA English

SL English

AB We set out to identify potential key regulators of exocytotic fusion in the eosinophil, in the knowledge that granule exocytosis can be stimulated in these cells by intracellular application of nonhydrolyzable analogues of guanosine triphosphate, with Ca2+ acting as a modulator of guanine nucleotide-dependent secretion. To screen for GTP-binding proteins, guinea pig eosinophils were purified from peritoneal washings and subjected to western blotting analysis using specific immune sera raised against recombinant proteins or consensus peptide sequences within proteins of interest. We found a number of heterotrimeric G proteins (G.alpha.(i3), G.alpha.0, G.alpha.11, G.alpha.(s) and G.beta. subunits) and members of the small GTP-binding proteins expressed in eosinophils. Two subtypes of G-protein alpha subunits (G.alpha.(i1) and G.alpha.(z)) could not be detected. Separation of subcellular organelles from homogenized eosinophils by density gradient centrifugation revealed that all of the detected GTP-binding proteins were mainly expressed in fractions containing peak plasma membrane and Golgi marker enzyme

activities, while G.beta. subunits were also detected in secretory granule fractions. However, isoforms of Rab3, a putative GTF-binding regulator of exocytotic fusion, were undetectable in eosinophils. Neither, with the exception of syntaxin-3, could we detect any of the proteins belonging to the proposed synaptic vesicle fusion complex (SNAP-25; **synaptobrevin** (**VAMP**) and its non-neuronal homologue, **cellubrevin**; synaptophysin; synaptotagmin). The results from this study, based on western blotting, suggest that eosinophils express a different class of exocytotic fusion complex proteins from those found in neuronal tissues, although a number of potential candidates fulfilling the role of G(E) were identified in this important inflammatory cell.

L26 ANSWER 18 OF 25 MEDLINE
AN 95138189 MEDLINE
DN 95138189
TI Synaptic core complex of **synaptobrevin**, syntaxin, and SNAP25 forms high affinity alpha-SNAP binding site.
AU McMahon H T; Sudhof T C
CS Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas 75235..
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Feb 3) 270 (5) 2213-7.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199505
AB SNAPs (soluble NSF attachment proteins) are cytoplasmic proteins that bind to specific membrane receptors and mediate the membrane binding of NSF (N-ethylmaleimide-sensitive factor), a protein that is required for membrane fusion reactions. Three synaptic proteins in brain (SNAP25 (synaptosomal-associated protein of 25 kDa; no relation to the SNAPs for NSF), **synaptobrevin/VAMP** , and syntaxin) were identified as SNAP receptors by affinity chromatography on immobilized alpha-SNAP complexed to NSF (Sollner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J. E. (1993) Nature 362, 318-324). However, the nature of the alpha-SNAP binding site is unclear. We now show that alpha-SNAP binds tightly to the complex of syntaxin with **synaptobrevin**. SNAP25 is not required for tight binding of alpha-SNAP to this complex but stabilizes the syntaxin-**synaptobrevin** complex by forming a trimeric core complex with it. alpha-SNAP does not bind to **synaptobrevin** individually and binds only weakly to syntaxin and SNAP25 in the absence of **synaptobrevin**. These data suggest that the complex of the vesicular protein **synaptobrevin** with the plasma membrane protein syntaxin is required for physiological alpha-SNAP binding. Thus, alpha-SNAP probably functions in a late step of the membrane fusion reaction after the formation of the **synaptobrevin**-syntaxin-SNAP25 core complex.

L26 ANSWER 19 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 8
AN 95038040 EMBASE
TI **Synaptobrevin** binding to synaptophysin: A potential mechanism for controlling the exocytotic fusion machine.
AU Edelmann L.; Hanson P.I.; Chapman E.R.; Jahn R.
CS Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, United States
SO EMBO Journal, (1995) 14/2 (224-231).
ISSN: 0261-4189 CODEN: EMJODG
CY United Kingdom
DT Journal
FS 003 Endocrinology

008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB The synaptic vesicle protein **synaptobrevin** (**VAMP**) has recently been implicated as one of the key proteins involved in exocytotic membrane fusion. It interacts with the synaptic membrane proteins syntaxin I and synaptosome-associated protein (SNAP)-25 to form a complex which precedes exocytosis. Here we demonstrate that the majority of **synaptobrevin** is bound to the vesicle protein synaptophysin in detergent extracts. No syntaxin I was found in this complex when synaptophysin-specific antibodies were used for immunoprecipitation. Conversely, no synaptophysin was associated with the **synaptobrevin** - syntaxin I complex when syntaxin-specific antibodies were used for immunoprecipitation. Thus, the **synaptobrevin** pool bound to synaptophysin is not available for binding to syntaxin I and SNAP-25, and vice versa. **Synaptobrevin**-synaptophysin binding was also demonstrated by chemical cross-linking in isolated nerve terminals. Furthermore, recombinant **synaptobrevin** II efficiently bound synaptophysin and its isoform synaptoporin, but not the more distantly related synaptic vesicle protein p29. Recombinant **synaptobrevin** I bound with similar efficiency, whereas the non-neuronal isoform **cellubrevin** displayed a lower affinity towards synaptophysin. Treatment with high NaCl concentrations resulted in a dissociation of the **synaptobrevin**-synaptophysin complex. In addition, the interaction of **synaptobrevin** with synaptophysin was irreversibly abolished by low amounts of SDS, while the interaction with syntaxin I was enhanced, We conclude that synaptophysin selectively interacts with **synaptobrevin** in a complex which excludes the t-SNAP receptors syntaxin I and SNAP-25, suggesting a role for synaptophysin in the control of exocytosis.

L26 ANSWER 20 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 9
AN 94206328 EMBASE
TI Cleavage of members of the **synaptobrevin/VAMP** family by types D and F botulinal neurotoxins and tetanus toxin.
AU Yamasaki S.; Baumeister A.; Binz T.; Blasi J.; Link E.; Cornille F.; Roques B.; Fykse E.M.; Sudhof T.C.; Jahn R.; Niemann H.
CS Department of Microbiology, Federal Virus Animals Dis. Res. Ctr., P. O. Box 1149, D-72001 Tubingen, Germany, Federal Republic of
SO J. BIOL. CHEM., (1994) 269/17 (12764-12772).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 004 Microbiology
LA English
SL English
AB Tetanus toxin (TeTx) and the various forms of botulinal neurotoxins (BoNT/A to BoNT/G) potently inhibit neurotransmission by means of their L chains which selectively proteolyze synaptic proteins such as **synaptobrevin** (TeTx, BoNT/B, BoNT/F), SNAP-25 (BoNT/A), and syntaxin (BoNT/C1). Here we show that BoNT/D cleaves rat **synaptobrevin** 1 and 2 in toxified synaptosomes and in isolated vesicles. In contrast, **synaptobrevin** 1, as generated by in vitro translation, is only a poor substrate for BoNT/D, whereas this species is cleaved by BoNT/F with similar potency. Cleavage by BoNT/D occurs at the peptide bond Lys59-Leu60 which is adjacent to the BoNT/F cleavage site (Gln58-Lys59) and again differs from the site hydrolyzed by TeTx and BoNT/B (Gln76-Phe77). **Cellubrevin**, a recently discovered isoform expressed outside the nervous system, is efficiently cleaved by all three toxins examined. For further characterization of the substrate

requirements of BoNT/D, we tested amino- and carboxyl-terminal deletion mutants of **synaptobrevin** 2 as well as synthetic peptides. Shorter peptides containing up to 15 amino acids on either side of the cleavage site were not cleaved, and a peptide extending from Arg47 to Thr116 was a poor substrate for all three toxins tested. However, cleavability was restored when the peptide is further extended at the NH2 terminus (Thr27-Thr116) demonstrating that NH2 terminally located sequences of **synaptobrevin** which are distal from the respective cleavage sites are required for proteolysis. To further examine the isoform specificity, several mutants of rat **synaptobrevin** 2 were generated in which individual amino acids were replaced with those found in rat **synaptobrevin** 1. We show that a Met46 to Ile46 substitution drastically diminishes cleavability by BoNT/D and that the presence of Val76 instead of Gln76 dictates the reduced cleavability of **synaptobrevin** isoforms by TeTx.

L26 ANSWER 21 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 10
AN 95015508 EMBASE
TI Identification of synaptic proteins and their isoform mRNAs in compartments of pancreatic endocrine cells.
AU Jacobsson G.; Bean A.J.; Scheller R.H.; Juntti-Berggren L.; Deeney J.T.; Berggren P.-O.; Meister B.
CS Berzelius Laboratory, Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden
SO Proceedings of the National Academy of Sciences of the United States of America, (1994) 91/26 (12487-12491).
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal
FS 003 Endocrinology
029 Clinical Biochemistry
LA English
SL English
AB Several proteins that are of importance for membrane trafficking in the nerve terminal have recently been characterized. We have used Western blot and immunohistochemistry to show that synaptotagmin, **synaptobrevin/VAMP** (vesicle-associated membrane protein), SNAP-25 (synaptosomal-associated protein of 25 kDa), and syntaxin proteins are present in cells of the islets of Langerhans in the endocrine pancreas. Synaptotagmin-like immunoreactivity (-LI) was localized to granules within the cytoplasm of a few endocrine cells located in the periphery of the islets, identified as somatostatin-containing cells, and in many nerve fibers within the islets. **VAMP**-LI was seen in granules of virtually all pancreatic islet cells and also in nerve fibers. SNAP-25-LI and syntaxin-LI were predominantly present in the plasma membrane of the endocrine cells, including insulin-producing .beta. cells. In situ hybridization, using isoform-specific oligonucleotide probes, detected **VAMP**- 2, **cellubrevin**, SNAP-25, syntaxin 1A, 4, and 5, and munc-18 mRNAs in isolated pancreatic islets and in insulin-producing cells. The results show the presence of several synaptic proteins at protein and mRNA levels in pancreatic islet cells, suggesting that they may have specific roles in the molecular regulation of exocytosis also in insulin-secreting cells.

L26 ANSWER 22 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 11
AN 94166806 EMBASE
TI Tetanus toxin-mediated cleavage of **cellubrevin** impairs exocytosis of transferrin receptor-containing vesicles in CHO cells.
AU Galli T.; Chilcote T.; Mundigl O.; Binz T.; Niemann H.; De Camilli P.
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University School of Medicine, 295 Congress Avenue, New Haven, CT 06510, United States
SO J. CELL BIOL., (1994) 125/5 (1015-1024).
ISSN: 0021-9525 CODEN: JCLBA3
CY United States
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB **Cellubrevin** is a member of the **synaptobrevin/VAMP** family of SNAREs, which has a broad tissue distribution. In fibroblastic cells it is concentrated in the vesicles which recycle transferrin receptors but its role in membrane trafficking and fusion remains to be demonstrated. **Cellubrevin**, like the synaptic vesicle proteins synaptobrevins I and II, can be cleaved by tetanus toxin, a metallo-endoprotease which blocks neurotransmitter release. However, nonneuronal cells are unaffected by the toxin due to lack of cell surface receptors for its heavy chain. To determine whether **cellubrevin** cleavage impairs exocytosis of recycling vesicles, we tested the effect of tetanus toxin light chain on the release of preinternalized transferrin from streptolysin-O-perforated CHO cells. The release was found to be temperature and ATP dependent as well as NEM sensitive. Addition of tetanus toxin light chain, but not of a proteolytically inactive form of the toxin, resulted in a partial inhibition of transferrin release which correlated with the toxin- mediated cleavage of **cellubrevin** . The residual release of transferrin occurring after complete **cellubrevin** degradation was still ATP dependent. Our results indicate that **cellubrevin** plays an important role in the constitutive exocytosis of vesicles which recycle plasmalemma receptors. The incomplete inhibition of transferrin release produced by the toxin suggests the existence of a **cellubrevin** -independent exocytotic mechanism, which may involve tetanus toxin-insensitive proteins of the **synaptobrevin/VAMP** family.

L26 ANSWER 23 OF 25 CAPLUS COPYRIGHT 1998 ACS
AN 1994:291492 CAPLUS
DN 120:291492
TI Inhibition of neurotransmitter release by tetanus and botulinum neurotoxins
AU Mochida, Sumiko
CS Dep. Physiol., Tokyo Med. Coll., Tokyo, 160, Japan
SO Seikagaku (1994), 66(3), 254-9
CODEN: SEIKAQ; ISSN: 0037-1017
DT Journal; General Review
LA Japanese
AB A review with 16 refs. on double-stranded structures, functions of each fragment, cloning of genes, identification of active sites, and functions as proteases in nerve ending of neurotoxins produced by Clostridium tetani and C. botulinum. Target mol. (e.g. **VAMP /synaptobrevin**, **cellubrevin**, SNAP-25, and syntaxin) of the neurotoxins are described.

L26 ANSWER 24 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 12
AN 94362146 EMBASE
TI Expression of vesicle-associated membrane protein 2 (**VAMP** -2)-**synaptobrevin** II and **cellubrevin** in rat skeletal muscle and in a muscle cell line.
AU Volchuk A.; Mitsumoto Y.; He L.; Liu Z.; Habermann E.; Trimble W.; Klip A.
CS Division of Cell Biology, Hospital for Sick Children, 555 University

Avenue, Toronto, Ont. M5G 1X8, Canada
SO BIOCHEM. J., (1994) 304/1 (139-145).
ISSN: 0264-6021 CODEN: BIJOAK
CY United Kingdom
DT Journal
FS 008 Neurology and Neurosurgery
029 Clinical Biochemistry
LA English
SL English
AB Molecular studies have identified a family of synaptic vesicle-associated membrane proteins (VAMPs, also known as synaptobrevins) which have been implicated in synaptic vesicle docking and/or fusion with plasma membrane proteins. Here we demonstrate the expression of two members of this family, **VAMP**-2/**synaptobrevin** II and **cellubrevin**, in skeletal muscle, a tissue with both constitutive and regulated membrane traffic. The 18 kDa **VAMP**-2 polypeptide was detected in purified membrane fractions from adult skeletal muscle and from L6 myotubes in culture, demonstrating that the presence of this protein in the isolated muscle membrane fractions is not the result of contamination by ancillary tissues such as peripheral nerve. Furthermore, skeletal muscle and the muscle cell line also expressed **cellubrevin**, a **VAMP**-2 homologue of 17 kDa, which is much less abundant in brain cells. Both **VAMP** -2 and **cellubrevin** were preferentially isolated in membrane fractions rich in plasma membranes, and were less concentrated in light microsomes and other internal membrane fractions of mature muscle or muscle cells in culture. Interestingly, both **VAMP**-2 and **cellubrevin** were much more abundant in the differentiated L6 myotubes than in their precursor myoblasts, suggesting that they are required for functions of differentiated muscle cells. The identity of both polypeptides was further confirmed by their susceptibility to proteolysis by Clostridium tetanus toxin. Expression of these products was further established by the presence of mRNA transcripts of **VAMP**-2 and **cellubrevin**, but not of **VAMP**-1, in both skeletal muscle and L6 myotubes. In contrast, other synaptic vesicle and docking/fusion components were undetectable, such as **VAMP**-1, SNAP25 and syntaxin 1A/1B, as were synaptophysin and synapsin Ia/Ib, proteins which are believed to be involved in sensing the signal for neuronal exocytosis. It is concluded that **VAMP**-2 and **cellubrevin** are expressed in skeletal muscle cells and may each participate in specific processes of intracellular membrane traffic.

L26 ANSWER 25 OF 25 EMBASE COPYRIGHT 1998 ELSEVIER SCI. B.V.DUPLICATE 13
AN 93265291 EMBASE
TI **Cellubrevin** is a ubiquitous tetanus-toxin substrate homologous to a putative synaptic vesicle fusion protein.
AU McMahon H.T.; Ushkaryov Y.A.; Edelmann L.; Link E.; Binz T.; Niemann H.; Jahn R.; Sudhof T.C.
CS Department of Molecular Genetics, Howard Hughes Medical Institute, Texas University SW Medical Center, Dallas, TX 75235, United States
SO NATURE, (1993) 364/6435 (346-349).
ISSN: 0028-0836 CODEN: NATUAS
CY United Kingdom
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English
AB TETANUS toxin inhibits neurotransmitter release by selectively blocking fusion of synaptic vesicles. Recently tetanus toxin was shown to proteolytically degrade **synaptobrevin** II (also named **VAMP**-2), a synaptic vesicle-specific protein, in

vitro and in nerve terminals. As targets of tetanus toxin, synaptobrevins probably function in the exocytotic fusion of synaptic vesicles. Here we describe a new **synaptobrevin** homologue, **cellubrevin**, that is present in all cells and tissues tested and demonstrate that it is a membrane trafficking protein of a constitutively recycling pathway. Like **synaptobrevin** II, **cellubrevin** is proteolysed by tetanus toxin light chain in vitro and after transfection. Our results suggest that constitutive and regulated vesicular pathways use homologous proteins for membrane trafficking, probably for membrane fusion at the plasma membrane, indicating a greater mechanistic and evolutionary similarity between these pathways than previously thought.